British Journal of Pharmacology

July 1994

Volume 112 Proceedings Supplement 442P–661P

1P A COMPARATIVE STUDY OF THE INFLUENCE OF ALLOSTERIC MODULATORS UPON HUMAN ($\alpha_3\ \beta_1\ \gamma_{2L}$) AND *DROSOPHILA* RECOMBINANT GABA RECEPTORS

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Numerous structurally dissimilar compounds are known to act as positive allosteric modulators of mammalian $GABA_A$ receptors (Siegart, 1992). By contrast, the susceptibility of invertebrate GABA receptors to allosteric regulation is less well defined. Here, the influence of the general anaesthetics propofol and etomidate, the neurosteroid 5α-pregnan-3α-ol-20-one (5α3α) and an isomer, δ-hexachlorocyclohexane (δ-HCH), of the insecticide lindane upon recombinant human and *Drosophila* GABA receptors expressed in *Xenopus laevis* oocytes has been examined. Data are reported as the mean ± s.e.m.

Oocytes were injected with either cRNAs encoding human α_3, β_1 and γ_{2L} $GABA_A$ receptor subunits, or a cRNA encoding a recently cloned *Drosophila melanogaster* receptor subunit (Belelli *et al*, 1994). 2 to 12 days later, GABA-evoked chloride currents were recorded under voltage-clamp at a holding potential of -60 mV. Current responses elicited by GABA demonstrated EC_{50} values of 101 ± 18μM (n = 4) and 151 ± 10μM (n=5) at human and *Drosophila* receptors respectively. Employing the appropriate GABA EC_{50}, picrotoxin exerted a potent (IC_{50} = 46 ± 4.6nM; n = 4) blockade of the *Drosophila* receptor, but was less effective upon the human recombinant receptor (IC_{50} = 2.2 ± 0.2μM; n=4). The effect of modulatory drugs was examined against the appropriate GABA EC_{10}. At the human and *Drosophila* GABA receptors respectively, propofol, in the ranges 0.3 - 30μM and 0.3 - 100μM, concentration-dependently increased the current response to GABA. Maximal potentiation, to 932 ± 69% of control (n=3) and 879 ± 138% of control (n=4), were observed with 30 and 100μM propofol acting at human and *Drosophila* receptors respectively. By contrast, etomidate (30μM) was inactive at the *Drosophila* receptor (n=3), but increased the response mediated by the human recombinant to 699 ± 88% of control (n=3). δ-HCH (100nM - 30μM) preferentially augmented currents mediated by the *Drosophila* receptor, maximal potentiations of 1090% (n=2) and 467 ± 37% (n=3) of control being found for the insect and human receptor respectively. The converse selectivity was observed with 5α3α, which potently enhanced human GABA responses (to a maximum of 520 ± 17% of control over the concentration range 1nM - 1μM; n=3), but produced a comparatively modest effect (maximal potentiation 206 ± 16% of control; n=5) when applied at 1 - 10μM to the *Drosophila* receptor. The *Drosophila* subunit exhibits a unique pharmacological profile and functions efficiently as a homo-oligomer. It may thus prove useful in mutagenesis studies which aim to define drug binding sites.

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2P $GABA_A$ RECEPTOR SUBUNIT mRNAS AND [^{3}H]SR 95531 BINDING SITES IN THE LOCUS COERULEUS

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Neuronal excitability in the locus coeruleus (LC) is controlled by GABA. In the present investigation we have examined the cellular expression patterns of 13 $GABA_A$ receptor subunit variants (α1-6, β1-3, γ1-3 and δ) in the rat LC by in situ hybridization histochemistry (Persohn et al., 1992) and the localization of the binding sites for different components of the $GABA_A$ receptor complex and for monoamine oxidases in the rat and human LC by receptor/enzyme radioautography (Saura et al., 1992).

Rat LC cells express mRNAs encoding the α3, β3 > α2,β1 > γ1 $GABA_A$ receptor subunits whereas other transcripts were not detected. Both rat and human LC cells have a high density of binding sites for the pyridazinyl-GABA derivative (^{3}H)SR 95531 (gabazine, a $GABA_A$ receptor antagonist for low affinity GABA recognition sites). However, at the concentrations used, no binding sites were detectable in the LC for the benzodiazepine receptor antagonist (^{3}H)flumazenil, the $GABA_A$ receptor agonist (for high affinity sites) (^{3}H)muscimol, or for the ionophore ligand (^{35}S)t-butyl bicyclophosphorothionate (TBPS). Unexpectedly, the pharmacological specificity of (^{3}H)SR 95531 binding to the LC differed markedly from that to most brain areas (IC50 for GABA and RU 5135 respectively in LC: > 10^{-2} M and 10^{-3} M; and in the dentate gyrus: $8{\cdot}10^{-7}$ M and $1.8{\cdot}10^{-9}$ M). Further characterization of (^{3}H)SR 95531 binding in the LC revealed a significant affinity for monoamine oxidase type A (MAO-A) which is highly concentrated in this nucleus. Inhibitors of MAO-A but not MAO-B, at concentrations which produce maximum but selective enzyme inhibition, competitively inhibited (^{3}H)SR 95531 binding (by up to 25%). Correspondingly, 2 h after the oral administration of supramaximal doses of the MAO-A inhibitors moclobemide and Ro 41-1049 (but not the MAO-B inhibitor lazabemide) the *in vitro* binding of (^{3}H)SR 95531 was markedly reduced (by 77 and 82%, of controls, respectively). Moreover, enzyme radioautography with (^{3}H)Ro 41-1049 revealed that SR 95531 has a significant affinity for MAO-A but not for MAO-B.

Altogether, these findings suggest that $GABA_A$ receptors in the LC are composed of a unique combination of subunits. Surprisingly, however, the high-affinity binding of (^{3}H)SR 95531 to the LC mainly reflects its affinity for MAO-A, which questions its utility as a selective ligand for low-affinity GABA recognition sites in the CNS.

Whether the identification of this potential drug target as well as the dual pharmacological profile of SR 95531, producing both disinhibition and potentiation of noradrenergic transmission in LC neurons, can be exploited as a rational for the development of new anxiolytics or antidepressants remains to be seen.

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3P DO BENZODIAZEPINE (ω) RECEPTOR PARTIAL AGONISTS DISRUPT THE ACQUISITION OF LEARNED FEAR IN MICE?

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It is well known that benzodiazepines can interfere with long term memory formation in humans. In experimental animals these drugs disrupt the acquisition of passive avoidance conditioning and spatial learning. There has been considerable discussion as to whether the effects of benzodiazepines on learning are related to their sedative actions. One way to investigate this question is to study the effects of BZ (ω) receptor partial agonists which are generally considered to produce certain effects associated with full agonists (eg anticonvulsant and anxiolytic actions) without being sedative. However, the available literature shows that while some partial agonists (bretazenil, U-78875, DN 2327) do not affect learning others (CGS 9896, ZK 91296) do. The present study investigated the effects of three partial agonists (bretazenil, Ro 171612 and Ro 198022) in a test of fear conditioning in mice previously used to evaluate a variety of BZ (ω) receptor ligands including CGS 9896 (Sanger and Joly 1985). The effects of the same drugs in increasing latencies of isoniazid-induced convulsions was used as an indication of intrinsic activity.

Effects of BZ(ω) Receptor Ligands on Fear Conditioning and Exploration in a Two Trial Passive Avoidance Procedure and on Isoniazid-induced Convulsions in Mice

Compound	Fear Conditioning	Exploration	Isoniazid Convulsions
	MED (mg/kg)		Latency (min)
Chlordiazepoxide	5	10	68
Bretazenil	> 10	> 10	39
Ro 171612	1	> 10	40
Ro 198022	1	> 10	31

The table shows that fear conditioning was disrupted by chlordiazepoxide, Ro 171612 and Ro 198022. Bretazenil had no effect, however, up to the dose of 10 mg/kg. Consistent with their partial agonist profile, bretazenil, Ro 171612 and Ro 198022 did not decrease locomotor activity (box crossing) during the exploratory phase of the two trial conditioning test. Partial agonism was also confirmed for these compounds in the anticonvulsant test. Bretazenil, Ro 171612 and Ro 198022 produced smaller maximum increases in the latency to isoniazid-induced convulsions than chlordiazepoxide.

The results show that BZ (ω) receptor partial agonists can interfere with learning without producing behavioural depressant activities indicating that with such drugs an effect on learning and memory is not invariably associated with sedation. However, not all partial agonists produce such an effect and differences between individual compounds are not clearly associated with different levels of intrinsic activity.

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4P IDENTIFICATION OF DISTINCT COMPONENTS, WITH DIFFERENT TIME COURSES, OF CONVULSION THRESHOLD CHANGES DURING ETHANOL WITHDRAWAL

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The severity of the ethanol withdrawal syndrome in mice is normally measured by ratings of the convulsive response to handling (Goldstein and Pal, 1971). The various symptoms in humans (tremors, convulsions, hallucinations) appear at different times. The present study investigated the timecourses of handling-induced convulsive behaviour, sensitivity to audiogenic seizures and sensitivity to different types of convulsant drugs, during ethanol withdrawal.

Male TO mice (20-30g) received ethanol by liquid diet (7 days at 24 g/kg/day). Control groups received the control diet in a pair-fed regime. All mice were withdrawn from ethanol at 09:00 hrs; treatment groups consisted of 10-12 mice. Handling-induced convulsive behaviour scores were rated once an hour for 24h on a scale of 0 to 5 (Littleton et al., 1990). The incidence of audiogenic convulsions was measured, in separate groups of mice, every 4h for 24h, using an electric door-bell rung for 30s. The threshold doses to produce a clonic convulsion were measured by intravenous infusion, every 4h in separate groups of mice, for 24h for the following convulsants: bicuculline, aminophylline, 4-aminopyridine (4-AP) and N-methyl-D,L-aspartate (NMDLA). All subjective ratings were made by an observer who was unaware of the prior drug treatment.

The ethanol withdrawn mice showed elevated scores for the responses to handling compared with control mice, with peak scores at 3-5h into withdrawal (median scores with interquartile ranges: 4h: ethanol = 2 (2-3); control = 0 (0-1)). The increases in audiogenic convulsions peaked at 8h into withdrawal (ethanol 6/10 compared with control 0/10, $P < 0.05$ Fisher's Exact Probability test). There was no change in the sensitivity to bicuculline at any of the intervals during the 24h ($P > 0.05$). The convulsion threshold to aminophylline was significantly elevated at the 4h time point (4h: ethanol = 1123 ± 72; control = 986 ± 48, mean ± s.e.m., $P < 0.05$ t-test). The convulsion threshold to 4-AP showed increases at 8h and 12h (12h: ethanol = 121 ± 8.3; control = 91 ± 8.3, mean ± s.e.m, $P < 0.05$) into withdrawal. The thresholds to NMDLA were significantly lowered later, with the peak change at the 16h time interval (ethanol = 233 ± 23; control = 302 ± 19, mean ± s.e.m., $P < 0.05$ t-test).

These data demonstrate that during ethanol withdrawal these convulsive responses all change, but with very different timecourses; the thresholds to aminophylline and to 4-AP were actually increased. The first changes were an increase in the convulsive responses to handling and an increase in the convulsive threshold to aminophylline. The lack of change in the thresholds to infused bicuculline indicate no role for changes in GABA sensitivity during ethanol withdrawal, as we have concluded previously (Whittington et al., 1992). The increases in the thresholds to 4-AP occurred later in the withdrawal period and may suggest previously undetected changes in potassium channels. The decreases in thresholds to NMDLA occurred after the handling and audiogenic responses had returned to control values, suggesting that this system may not be involved in these responses during ethanol withdrawal.

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5P ANTI-CONVULSANT EFFECTS OF TIAGABINE AND NNC-711 IN THE GENETICALLY EPILEPSY-PRONE RAT

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Reproducible clonic seizures may be induced in genetically epilepsy-prone (GEP) rats upon exposure to a sound stimulus (Faingold, 1988).
GABA-mimetics which act at the GABA receptor complex (diazepam) or by inhibition of the neuronal and/or glial uptake of γ-aminobutyric acid (GABA) ((±)-nipecotic acid (3-piperidinecarboxylic acid), tiagabine ((R-)-N-(4,4-di-(3-methylthien-2-yl)-but-3-enyl) nipecotic acid•HCl), and NNC-711 ((1-(2-(((diphenylmethylene)amino)oxy)-ethyl)-1,2,5,6-tetrahydro-3-pyridine-carboxylic acid•HCl)) (Suzdak *et al.*, 1992) were compared with carbamazepine for anticonvulsant efficacy and locomotor impairment in GEP rats.
Seizures were induced using a sound stimulus (100-110 dB, 60s). Locomotor performance was tested using a rod (9cm diameter with 1.5 mm deep grooves at 3° intervals) rotating at 12 r.p.m. for 60s. 1h later animals (250-500 g) received test compounds or vehicle (i.p.) and were tested repeatedly at the times after administration detailed in table 1. Results are expressed as ED_{50} values in μmol/kg for the dose of compound corresponding to 50% of the number of animals with protection from clonic seizure or impaired rotarod performance.
(±)-Nipecotic acid (0.4-15.6 x 10^3 μmol kg^{-1}) was not anticonvulsant. High doses of NNC-711 (207-310 μmol kg^{-1}) and of (±)-nipecotic acid (39-78 x 10^3 μmol kg^{-1}) induced ataxia and myoclonic seizures after 0.25-1 h. Tiagabine and carbamazepine had similar therapeutic indices (locomotor deficit ED_{50}/anticonvulsant ED_{50}) ranging from 0.4 to 1.9.

Table 1: ED50 values (μmol/kg) for antagonism of clonic seizures and for ataxia

Drug (m.w.)		Time after i.p. administration: Seizure testing						Rotarod testing						
		+15′	+30′	+1h	+2h	+4h	+8h	+7.5′	+15′	+30′	+1h	+2h	+4h	+8h
Diazepam	(285)	0.8	0.5	1.9	3.1	NDD	---	6.2	9.8	10	13	NDD	---	---
Carbamazepine	(236)	---	254	168	98	132	330	258	135	159	169	190	224	NDD
Tiagabine	(412)	37	23	26	41	56	61	17	13	16	16	41	66	NDD
NNC-711	(387)	NDD	NDD	72	131	202	238	31	20	21	46	172	297	---

---: Not tested, NDD: Not dose-dependent.

Tiagabine has comparable anticonvulsant action to carbamazepine in GEP rats supporting the potential use of inhibitors of GABA uptake as therapy for epilepsy.

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6P MODIFICATION OF SYNAPTIC RESPONSES OF ENTORHINAL CORTICAL NEURONES BY A NEW $GABA_B$ ANTAGONIST, CGP 55845

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$GABA_B$ receptors at both pre- and postsynaptic sites exert a widespread influence on synaptic transmission in the CNS (Bowery, 1993). We have been studying the part played by $GABA_B$ receptors in short-term frequency facilitation of synaptic transmission in the rat entorhinal cortex (EC) *in vitro*. Examination of the role of $GABA_B$-receptors has depended on the use of relatively weak antagonists such as phaclofen and 2-hydroxysaclofen. Recently, more potent antagonists have been developed which are some 200-500 times more effective than the earlier generation of compounds (Froestl *et al*, 1992). The present study describes the effects of one such compound, CGP 55845, on synaptic responses of neurones in layer V of the EC. Experiments were conducted in slices of rat EC maintained *in vitro* at the interface between artificial CSF (at 34°C) and carbogen gas (95% O_2, 5% CO_2). Conventional intracellular voltage recordings were made from neurones in layer V of the EC and synaptic responses were elicited by electrical stimulation of intracortical pathways.

Synaptic responses were complex and varied from cell to cell. They invariably displayed a fast, AMPA/kainate receptor-mediated EPSP. This was succeeded by a slow NMDA receptor-mediated depolarization in most cells. In others, fast and slow inhibitory components dominated the later parts of the response. A clear, slow IPSP was present in 7 neurones and this could be reduced or blocked by bath applied CGP 55845. At 0.5 μM, the drug reduced the amplitude of the slow IPSP from 6.4±1.1 mV (mean±s.e.m.) to 2.7±0.2 mV.(n=3) a reduction of 57%. Higher concentrations of CGP 55845 (1.0-10 μM, n=4) abolished the slow IPSP. The fast ($GABA_A$) IPSP was not reduced by CGP 55845. In fact, in a number of cells (n=5) it appeared to be enhanced. The fast AMPA-receptor mediated EPSP (n=14) was significantly reduced (-18±4.8 %, $P<0.05$, paired t-test) by CGP 55845. The mean amplitude of the control EPSPs was 10.9±0.8 mV compared to 8.9±0.9 mV after the addition of the antagonist. Finally, the duration of synaptic depolarizations was clearly increased by CGP 55845 (from 188±35 ms to 301±34 ms, n=8, $p<0.05$) largely as a result of a much prolonged slow component. This late component could be blocked by 2-AP5 (30 μM) and thus was mediated by NMDA receptors.

Thus, CGP 55845, proved to be a powerful and selective antagonist of the slow IPSP in the EC confirming its mediation via $GABA_B$-receptors. Blockade of the slow IPSPs allowed greater expression of the slow NMDA-mediated EPSP clearly showing the overlap of these components. The enhanced fast IPSPs occasionally observed may reflect a reduction of GABAergic inhibition of GABA interneurones. Fast EPSPs were reduced and this could result from the concurrent enhancement of the fast IPSPs or, alternatively, from an agonist effect of CGP 55845 at presynaptic $GABA_B$-receptors on glutamate terminals, such as has been suggested for 2-hydroxysaclofen (Caddick and Chad, 1993).

Acknowledgements: We thank the Wellcome Trust for financial support and Ciba-Giegy for CGP 55845. AD is an MRC Scholar

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7P NMDA RECEPTORS AND NO SYNTHASE (NOS) ARE INVOLVED IN THE INCREASE OF CEREBRAL cGMP INDUCED BY $GABA_B$ ANTAGONISTS

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We have shown that the selective $GABA_B$ receptor agonist R(-)-baclofen (1-10mg/kg i.p.) decreases cGMP levels in different brain structures of rodents in a dose-dependent manner (Bernasconi *et al.* , 1992). We now report that the centrally-active $GABA_B$ receptor antagonist CGP35348 (P-(3-aminopropyl)-P-diethoxymethyl-phosphinic acid) at 200mg/kg i.p. (n=3) failed to alter cerebellar cGMP levels but completely antagonized the decrease produced by (-)baclofen (4mg/kg). Higher doses of this antagonist (e.g. 400mg/kg i.p.) or of another with similar affinity for the $GABA_B$ receptor, CGP36742 (P-(3-aminopropyl)-P-n-butyl-phosphinic acid) did produce an increase (170% over basal; n=8) in cerebellar cGMP in the absence of (-)baclofen. However, dose-dependency was only observed after intracerebellar (icb) injection of CGP36742: at 3µg the increment was 174% and reached 228% at 10µg. Systemic administration of $GABA_B$ antagonists with affinities for the $GABA_B$ receptor in the low nanomolar range such as CGP55556 (lithium-3-{N-[1-(3-carboxyphenyl)-ethyl]amino}-2S-hydroxy-propyl(cyclohexylmethyl) phosphinate) or of its RS diastereoisomer CGP56999, elicited a dose-dependent enhancement of cerebral cGMP: at1 mg/kg CGP55556 the increase was 149%, at 3mg 245% and at 10mg 180% over basal values.

To test whether NMDA receptors were involved in the increase in cGMP induced by $GABA_B$ antagonists, we injected antagonists of the different binding sites of the NMDA receptor complex either alone or in combination with $GABA_B$ antagonists. The results showed that these effects of $GABA_B$ antagonists were abolished by CGP40116 (5mg/kg), ((2R)-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid), R(+)-HA966 (5mg/kg), or by spermidine (100µg/icb). The two NOS inhibitors N-monomethyl-L-arginine (25µg icb) and nitro-L-arginine (5µg icb) suppressed the increase in cerebellar cGMP produced by CGP36742 (10µg icb). Taken together, these results suggest that blockade of the $GABA_B$ receptors increases glutamate release, which depolarizes the postsynaptic membrane, reducing the blockade by Mg^{++} of NMDA channels to allow the entry of Ca^{++}. This activates NOS, via calmodulin, to form NO which in turn activates soluble guanylate cyclase. In support of this possibility, CGP56999 (6mg/kg) induced clonic seizures of the face and of the fore-limbs associated with epileptiform discharges on the EEG. These epileptic phenomena were antagonized by R(-)-baclofen (100% suppression at 4mg/kg; n=10) and by CGP40116 (94% reduction at 3mg/kg; n=10).

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8P FURTHER CHARACTERISATION OF GABAPENTIN BINDING SITES IN BRAIN

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Gabapentin (GBP), is a novel anticonvulsant with efficacy in humans (Chadwick, 1992). Its mechanism of action is unknown although a novel high affinity binding site for [^{3}H]GBP, unique to brain, may be involved (Suman-Chauhan et al., 1993). Of the compounds originally tested, only GBP and 3-substituted γ-amino-butyric acid (GABA) analogues possessed high affinity for this binding site. The binding site has a heterogeneous autoradiographic distribution in areas which are associated with major excitatory input (Hill et al., 1993). We now report that large neutral amino acids (LNAA) inhibit [^{3}H]GBP binding stereospecifically and with high affinity. The binding site may also be localized selectively on neurones.

Inhibition profiles of amino acids and related compounds were determined by competition with [^{3}H]GBP as described previously (Suman-Chauhan et al., 1993). Non-specific binding was determined using 10µM (S)-(+)-3-isobutyl GABA. Binding of [^{3}H]GBP was also performed using washed membranes prepared from primary cultures of glia and cerebellar granule cells from neonatal rats.

Competition studies using mouse brain membranes revealed that LNAA were potent, stereospecific displacers of [^{3}H]GBP binding. The results shown here represent the means of at least three separate experiments. L-leu (IC_{50}=77nM), L-isoleu (IC_{50}=72nM) ,L-norleu (IC_{50}=39nM) and L-met (IC_{50}=51nM) all inhibited binding with similar affinities to GBP itself (IC_{50}=50nM) while the D-enantiomers were less active (D-leu IC_{50}=154µM). L-val (IC_{50}=314nM), L-phe (IC_{50}=384nM) and the selective system-L substrate, 2-amino-2-carboxy-bicycloheptane (BCH) (IC_{50}=423nM), also inhibited but with lower affinity for the site than GBP. The sodium dependent system-A substrate (Christensen,1990), methyl-aminoisobutyric acid (MeAIB) and a range of acidic and basic amino acids, were inactive at the binding site at concentrations up to 1mM. Saturation analysis in the presence of L-leu (60nM) suggested a competitive interaction, with the amino acid significantly reducing [^{3}H]GBP binding affinity (Mann-Whitney, 2 sample test; one tailed, p=0.014) for the site rather than the maximum number of binding sites (control pK_D=7.37 ± 0.12, B_{max}= 7.63 ± 0.32 pmols/mg protein; +60nM L-leu pK_D=6.98 ± 0.06, B_{max}= 8.45 ± 1.10 pmols/mg protein). Binding of [^{3}H]GBP observed in washed cerebellar granule cell membranes (60% specific binding) but not in washed glia cell membranes, was displaceable by GBP (IC_{50}=122nM), (S)-(+)-3-isobutyl GABA (IC_{50}=202nM), L-leu (IC_{50}=123nM) and BCH (IC_{50}=477nM),. No specific [^{3}H]GBP binding was observed in washed membranes prepared from a number peripheral tissues (liver, spleen, kidney, lung, heart & pancreas)

This study shows that [^{3}H]GBP binding is inhibited competitively and stereoselectively by LNAA, the specific system-L inhibitor BCH, but the site is insensitive to the system-A inhibitor MeAIB. These results raise the possibility that the GBP binding site in brain may represent a component of a LNAA transporter (Christensen, 1990) centrally localised on a population of excitatory neurones.

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9P THE GLYCINE/NMDA RECEPTOR ANTAGONISTS, R-(+)-HA966 AND 5,7 DICHLOROKYNURENIC ACID ATTENUATE NALTREXONE-PRECIPITATED MORPHINE WITHDRAWAL IN THE RAT

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Previous studies have demonstrated that the non-competitive NMDA receptor antagonist, MK-801, attenuates naloxone precipitated behaviours in morphine-dependent rats (Trujillo and Akil, 1991). Given that at least 3 distinct classes of NMDA receptor antagonist exist we have examined the effects of the glycine/NMDA receptor antagonists, R-(+)-HA966 and 5,7-dichlorokynurenic acid on naltrexone precipitated morphine withdrawal in rat.

Male Sprague Dawley rats (250 - 350 g) were anaesthetised with isoflurane and subcutaneously implanted with a single morphine pellet (75 mg free base). Forty eight hours later, rats were habituated to single perspex observation boxes for at least 30 min and then pretreated with either R-(+)-HA966 (10-60 mg/kg, s.c., n=8/group) or 5,7-dichlorokynurenic acid (5,7-dichk; 5 µg/5µl, n=7/group) administered intracisternally (i.c.s) through a previously implanted catheter. Control animals received vehicle (0.9% saline, 1ml/kg (n=8)) or distilled water (5µl (n=7)) via the relevant injection route. Fifteen or 30 min later, all rats received naltrexone (1 mg/kg, s.c.) and behaviours recorded for the following 15 min. Results are expressed as either the mean number ± s.e. mean of wet dog shakes (WDS), ejaculations or the duration of teeth chattering (analysed by analysis of variance followed by Dunnett's t-test) or % animals showing a given response (ptosis, irritability and diarrhoea; analysed using Fishers exact probability test; * = P<0.05).

Compound	Dose	WDS	Irritability (%)	Diarrhoea (%)	Teeth chattering(sec)	Ejaculation	Ptosis (%)
Vehicle		10 ± 0.2	100	62.5	272 ± 35	3.0 ± 0.3	100
Clonidine	0.2 mg/kg	2 ± 0.4*	87.5	0.0*	156 ± 37*	1.8 ± 0.3*	25*
Vehicle		10 ± 1.9	100	87.5	181 ± 28	2.6 ± 0.4	100
(+)-HA-966	10 mg/kg	7 ± 1.8	100	37.5	161 ± 20	3.0 ± 0.3	62
	30	6 ± 1.4	87.5	12.5*	95 ± 23*	2.3 ± 0.4	50*
	60	3 ± 1.1*	87.5	0.0*	27 ± 7*	1.3 ± 0.3*	0*
Vehicle		21 ± 2.0	100	71.0	201 ± 38	1.4 ± 0.3	86
5,7 dichk	5 µg/rat	7 ± 2.0*	14*	14.0*	89 ± 23*	0.3 ± 0.2*	29*

Pretreatment with R-(+)-HA966 or 5,7 dichlorokyurenic acid, significantly attenuated naltrexone precipitated behaviours in morphine dependent rats.These results provide further evidence supporting a role for NMDA receptors in the expression of opiate withdrawal.

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10P NMDA-INDUCED GLUTAMATE RELEASE FROM RAT CORTICAL PYRAMIDAL NEURONES IS POTENTIATED BY A 5-HT_{1A} ANTAGONIST

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Hypoactivity of cortical glutamatergic pyramidal neurones may underlie the histopathological as well as the cognitive disorders found in Alzheimer's disease. Serotonin (5-$HT)_{1A}$ receptors appear to be enriched on these neurones (Francis et al., 1993). This study tests the functional significance of this enrichment in vivo using a slight modification of the method of Palmer et al (1989).

Male Sprague Dawley rats (200-250 g) were anaesthetised with halothane/O_2/N_2O. A microdialysis probe was implanted in the striatum (coordinates with bregma as reference: AP 0 mm, L 2.5 mm and DV 7 mm from skull). In order to be able to stimulate pyramidal neurone bodies in the cortex, the cranium was opened over the ipsilateral frontal cortex and the dura carefully removed. After a stabilisation period of 1 hour, 15 (10 minute, flow rate 1 µl/min) samples were collected. At the beginning of each sampling period the craniotomy was filled with phosphate buffered saline (PBS;10 µl). At the beginning of the seventh sample-period either PBS or drugs dissolved in PBS were applied. Samples were frozen at - 70 °C until assayed by HPLC with fluorescent detection following o-phthaldialdehyde/2-mercaptoethanol derivatisation. For each treatment the mean (± s.e.mean pmol glutamate in 10 µl) of the first six samples (baseline) was compared to the eleventh sample (maximal effect) using the Wilcoxon Matched-pairs Signed-rank test. N-methyl-D-aspartate (NMDA; 20 mM, n=6) caused a rise in glutamate release from 3.6 ± 0.6 to 12.0 ± 2.4 (p=0.028); 2mM NMDA (n=7) from 3.2 ± 0.4 to 7.8 ± 0.9 (p= 0.018). The 5-HT_{1A} antagonist (+)WAY 100,135 (Fletcher et al., 1993);50µM) potentiated the effect of 2mM NMDA when co-applied increasing release from 3.1 ± 0.3 to 14.3 ± 1.8 (n=13) (maximal effect significantly higher than for NMDA alone, Mann-Whitney U-test, p=0.01). Co-applied tetrodotoxin (10µM) inhibited the effect of 20mM NMDA by 80% and that of 2mM NMDA with 50µM (+)WAY 100,135 by 90%. Application of 50µM (+)WAY 100,135 alone caused a significant elevation of glutamate release from 2.8± 0.4 to 3.25 ± 0.21 (p=0.0052).

The present study confirms that topically applied NMDA increased release of glutamate in the striatum (Palmer et al., 1989), probably from corticostriatal pyramidal neurones. Since (+)WAY 100,135 potentiated the NMDA-mediated effect, 5-HT_{1A} antagonists may be useful to reverse the hypoactivity of cortical pyramidal neurones in Alzheimer's disease.

Supported by the Brain Research Trust, we thank Wyeth for (+)WAY 100,135.

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11P EFFECT OF LAMOTRIGINE ON GLUTAMATE AND SUBSTANCE P RELEASE FROM THE RAT SPINAL CORD

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The anticonvulsant lamotrigine has been shown to exhibit antinociceptive effects in the rat (Nakamura-Craig and Follenfant 1992) but the mechanism underlying this action is unclear. Since it has been proposed that inhibition of glutamate release within the brain may be responsible for the anticonvulsant action (Leach et al 1986) we wondered whether a similar mechanism within the spinal cord might contribute to the antinociceptive effect. In the present study, we have therefore, examined the effect of lamotrigine on the release of endogenous glutamate evoked by electrical stimulation of dorsal root afferents in rat isolated spinal cord. Comparison with its effect on the release of Substance P-like immunoreactivity (SP-LI) in the same tissue has also been performed. Hemisected dorsal horn slices with roots attached were placed in a three-compartment bath and continuously superfused with Krebs' solution at $1ml.min^{-1}$. Dorsal roots were positioned on bipolar platinum electrodes in each lateral compartment containing mineral oil. Consecutive three min (glutamate) or eight min (SP-LI) fractions were collected. In the glutamate studies, two stimuli (S1 and S2), each of 20V, 1Hz and 0.5msec were applied for three minutes separated by an interval of nine minutes. The glutamate content in each sample was determined by HPLC with o-phthaldialdehyde precolumn derivatisation and fluorimetric detection. For SP-LI studies, only one eight min stimulation (same parameters) was applied. SP-LI was detected by radioimmunoassay (Malcangio and Bowery 1993). Statistical analyses were performed using ANOVA and Dunnett's test.

The mean basal concentration of glutamate in perfusates from control slices was 216.6 ± 22.5nM (n = 4) with mean stimulated S1 and S2 values of 217.8 ± 16.6% ($p < 0.01$) and 184.3 ± 3.0% ($p < 0.05$) of basal respectively (S2/S1 ratio = 0.706). Lamotrigine (0.1μM - 300μM) had no effect on the basal level but produced a significant dose-dependent decrease ($p < 0.05$ at 10μM and above) in the S2/S1 value (e.g. 0.41 ± 0.06 at 300μM; n = 3) when present during the S2 period. The stimulated release of SP-LI from control slices was 228 ± 59% of basal (mean basal level = 1.0 ± 0.06nM; n = 3). Addition of lamotrigine to the bathing solution (300μM) produced no significant effect on either the basal or stimulated release of SP-LI (248 ± 74%; n = 3).

We conclude that inhibition of evoked glutamate release in spinal cord may contribute to the antinociceptive effect of lamotrigine. Whilst the lack of effect on Substance P release fails to implicate this neuropeptide as well, it does support the specificity of lamotrigine for the glutamate system.

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12P *IN VIVO* AND *IN VITRO* ELECTROPHYSIOLOGICAL ACTIONS OF NOVEL 2,3-BENZODIAZEPINE AMPA ANTAGONISTS

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The 2,3-benzodiazepine, GYKI 52466, a selective inhibitor of AMPA receptor mediated excitation, has anticonvulsant, central muscle relaxant and neuroprotective actions (Tarnawa *et al.*, 1989; Smith *et al.*, 1991; Smith & Meldrum, 1992). Testing of recently synthesised novel analogues of GYKI 52466 showed that introduction of certain substituents into position 3 of the benzodiazepine ring resulted in increased potency (Tarnawa *et al.*, 1993; Palmer & Lodge, 1993). In the present study, some electrophysiological actions of 3-acetyl and 3-methyl-carbamoyl derivatives are described. Effects on flexor reflexes were investigated in chloralose anesthetised cats by electrically stimulating one hind paw and recording responses from the anterior tibial muscle myographically (Farkas & Karpati, 1988). Effects on CA1 field potentials were examined in rat hippocampal slices by stimulating the Schaffer collateral-commissural pathway and recording from the stratum pyramidale and stratum radiatum at the same time (Tarnawa et al., 1993).

The 3-acetyl and 3-methylcarbamoyl derivatives of (±)-1-(4'-aminophenyl)-4-methyl-3,4-dihydro-7,8-methylenedioxy-2,3-benzodiazepine (ie. GYKI 53405 and GYKI 53655) were respectively 2.2 and 4.1 times more potent than GYKI 52466 in the flexor test (n=6) while, on the CA1 field potentials, the corresponding IC50 values were 1.2 and 2.6 times lower than that of GYKI 52466. In contrast to GYKI 52466, these compounds have an asymmetric centre in position 4 of the ring, the optical isomers of each compound have been prepared. The (-) isomers were more effective than their corresponding racemates both in vivo and in vitro. Their IC50 values for CA1 field potential inhibition (n>5) were 8.0 ± 1.4 and 2.5 ± 0.4uM respectively while the corresponding ED50 values in the flexor reflex test were 0.15 ± 0.10 (n = 7) and 0.06 ± 0.03 (n = 5) mg/kg intravenously. The (+) isomers were without effect. A more detailed analysis of the spinal reflex inhibitory action of GYKI 52466 and GYKI 53405 revealed that there were no qualitative differences between the actions of parent and substituted compounds on the various components of reflex potentials recorded from ventral and dorsal roots in spinal cats.

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13P STEREOSPECIFIC EFFECTS OF 2,3-BENZODIAZEPINE AS ANTAGONISTS OF α-AMINO-3-HYDROXY-5-METHYL-ISOXAZOLEPROPIONATE (AMPA)

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The 2,3-benzodiazepine, GYKI 52466, selectively blocks monosynaptic reflexes (Tarnawa *et al.*, 1989) and AMPA receptor mediated excitation of rat spinal neurones in vivo (Jones & Lodge, 1991) and cortical slices (Palmer, Zeman & Lodge, 1991). More potent 2,3-benzodiazepine analogues, GYKI 53405 and 53655, have acetyl and methylcarbamoyl substitutions respectively at the 3 position which results in an asymmetric carbon at position 4 (Tarnawa & Farkas, 1993). Hence we have investigated the effects of the separated isomers using iontophoretic techniques on spinal neurones of pentobarbitone-anaesthetised rats and bath application on cortical slices.

On 10 rat spinal neurones, (-) GYKI 53405 (4mM in 100mM NaCl; 3-10nA), reduced responses to AMPA by 91 ± 3% (mean ± s.e.m.) and those to NMDA by 3 ± 2% whereas the (+) isomer was without effect up to 40nA. Similarly given intravenously, the (-) isomer (5 mg/kg) reduced AMPA responses by 94 ± 3% (n = 9) whereas the (+) isomer (20mg/kg) was inactive. Similar stereoselectivity was seen with the (-) and (+) isomers of GYKI 53655. Recovery from both local and parenteral administration were rapid. Following intragastric perfusion of (-) GYKI 53405 10mg/kg (n = 6), responses to AMPA and NMDA were reduced by 68 ± 6% and 9 ± 2% respectively, with partial recovery in 1 hour.

On rat cortical wedges, superfusion of the (-) isomers of GYKI 53405 and 53655 resulted in a selective, dose-dependent and reversible inhibition of responses to AMPA but not of NMDA or kainate, the IC50 values versus 40uM AMPA being 6 ± 1 and 2.5 ± 2 uM respectively. Recovery was obtained within one hour of return to control solutions. Dose-response curves for AMPA were shifted in a non-parallel manner suggesting non-competitive antagonism. The epileptiform activity seen in these cortical slices in low magnesium was reduced by the (-) isomers of the 2,3 benzodiazepines. Thus 10uM (-) GYKI 53655 reduced both burst frequency and duration by about 50% whereas the (+) isomer was inactive. Each of these observations were made on at least 4 slices.

These results indicate that the activity of racemic mixtures of the 2,3-benzodiazepines resides in the (-) isomer and that these compounds are active as AMPA antagonists following local and systemic administration.

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14P THE EFFECT OF ANIRACETAM AND CYCLOTHIAZIDE ON THE ANTICONVULSANT ACTION OF FOUR AMPA ANTAGONISTS IN RODENT SEIZURE MODELS

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We have previously shown that the AMPA antagonists, NBQX and GYKI 52466 protect against sound-induced and AMPA-induced seizures in rodents (Chapman et al., 1991; Smith et al., 1991), and that aniracetam reverses the anticonvulsant action of GYKI (Chapman et al. 1993). We are extending our anticonvulsant studies to the novel AMPA antagonists, LY 293558 ((3S,4αR,6R,8αR)-6-[2(1H-tetrazol-5-yl)ethyl]decahydro-isoquinoline-3-carboxylic acid) (Ornstein et al., 1993), and the quinoxaline-dione, YM900. We have also assessed the ability of aniracetam and cyclothiazide to reverse the anticonvulsant protection of the AMPA antagonists; both compounds are known to enhance the depolarizing action of AMPA. Sound-induced clonic seizures in DBA/2 mice and GEP rats, AMPA-induced seizures in Swiss mice, and rotarod performance were assessed as previously described (Chapman et al., 1991; Smith et al., 1991). Table 1 compares the ED_{50} values of 4 AMPA antagonists in 3 seizure models assessed at 30 min, when all drugs showed optimal anticonvulsant activity. LY 293558 is the most potent in all seizure models; the antagonists vary with respect to rotarod impairment; the aniracetam reversal of anticonvulsant potency is relatively specific for the noncompetitive AMPA antagonist GYKI, while cyclothiazide affects none of the antagonists.

Table 1.
Anticonvulsant ED_{50} values (ip, 30 min, μmol/kg) of AMPA antagonists against sound-induced clonic seizures in DBA/2 mice and GEP rats and against AMPA-induced seizures in Swiss mice; the effect of aniracetam (50 nmol icv, 30 min) and cyclothiazide (13 nmol icv, 30 min) on the anticonvulsant potency in DBA/2, and TI ratios assessed by rotarod performance in DBA/2.

	GEP rats	AMPA-seizures	DBA/2 mice (antag. alone)	DBA/2 mice (+ anirac.)	DBA/mice (+ cyclothi.)	TI ratio (DBA/2)
NBQX	67 (14-322)	24 (12-48)	40 (34-47)	86 (64-115)	28 (21-36)	6.6
GYKI 52466	39 (17-92)	19 (12-30)	20 (12-34)	142 (92-219)	25 (19-32)	2.0
YM900	ND	60 (32-115)	52 (42-63)	58 (26-129)	43 (21-86)	1.1
LY 293558	3.9 (1.7-9.1)	ND	9.7 (7.5-13)	22 (19-27)	7.8 (5.2-12)	6.6

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15P RE-EVALUATION OF THE EFFECTS OF PUTATIVE COGNITIVE DISRUPTERS IN THE REINFORCED ALTERNATION T-MAZE TASK IN THE RAT

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The effects of four drugs were examined in the reinforced alternation T-maze task in the rat: the central and peripheral muscarinic antagonist scopolamine hydrobromide (HBr); the peripheral muscarinic antagonist scopolamine methylbromide (MeBr); the 5-HT_{1A} agonist 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and the non-competitive N-methyl-D-asparate (NMDA) antagonist MK-801. In this test, Hooded Lister rats were trained to remember the location of a previously visited arm of a T-maze in order to obtain food reward (see Salamone *et al.*, 1984). On test days, the rats (n=7-8) were pretreated s.c. with either saline or drug. The pretreatment time was 30 minutes for scopolamine and MK-801 and 3 minutes for 8-OH-DPAT. Drug testing occurred no more frequently than once per week. Eight choice trials occurred during test. The percentage of correct choices and the latency to consume the food pellets during the 8 trials were recorded and the mean values are shown in the Table below. Data analysis was conducted using one- and two-way analysis of variance (ANOVA) and, when appropriate, post-hoc Student Newman Keuls tests.

Drug (mg/kg base)	% Correct	Latency (s)	Drug (mg/kg base)	% Correct	Latency (s)
Saline	95±3.7	15±4.1	Saline	83±6.3	16±2.7
Scopolamine HBr 0.05	75±10.6*	34±11.1	8-OH-DPAT 0.03	95±3.4	20±4.4
Scopolamine HBr 0.10	67±6.6*	52±12.2*	8-OH-DPAT 0.10	74±8.9	39±7.0
Scopolamine MeBr 0.10	98±1.8	17±4.3	8-OH-DPAT 0.30	41±12.4*	70±11.6*
Saline	88±3.2	7±0.8	Saline	89±5.7	18±3.9
MK-801 0.025	89±5.1	8±1.1	MK-801 0.10	55±7.6*	28±6.0
MK-801 0.05	92±4.2	10±1.6			
MK-801 0.10	49±9.7*	49±10.8*	*=significantly different from saline control ($p<0.05$)		

Scopolamine HBr induced a significant decrease in the percentage of correct choices and also increased the latency to consume food pellets. Scopolamine MeBr had no effect on either of these measures. 8-OH-DPAT impaired both the percentage of correct choices and the latency to complete trials. MK-801 also induced cognitive impairments and significantly increased the latency to consume pellets. Further statistical analysis, however, revealed that the increase in latency induced by MK-801 was significant only during the first half of the test session, whereas the cognitive impairment was constant throughout the session. This was particularly apparent in the second experiment in which the increase in latency induced by MK-801 was only observed on the first two trials. Taken together these results suggest: 1) the apparent cognitive disrupting effects of scopolamine HBr and 8-OH-DPAT may be due to non-specific side-effects; 2) the nonspecific side-effects induced by scopolamine HBr appear to be centrally mediated; 3) MK-801 has cognitive disrupting effects in this test and these effects do not appear to be secondary to an increase in latency to perform the task.

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16P A COMPARISON OF THE EFFECTS OF ENADOLINE AND DIZOCILPINE ON IMMEDIATE EARLY GENE EXPRESSION IN THE CENTRAL NERVOUS SYSTEM

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The drug dizocilpine has a neuroprotective role in models of ischaemia by its actions as a non-competitive NMDA antagonist. However, it possesses other actions which potentially limit its therapeutic usefulness. Thus, it is capable of greatly increasing the metabolic activity of neurones in the limbic system as measured by 2-deoxyglucose metabolism (Nehls et al 1988) and, more recently, it was demonstrated to stimulate the expression of immediate early genes (IEGs) in these same areas (Dragunow & Faull 1990; Gass et al 1993). A similar increase in IEG expression has been observed with ketamine and phencyclidine; these changes have been ascribed to the pharmacological profile of psychomimetic drugs. This report compares the ability of dizocilpine to stimulate IEG expression with that of the kappa agonist enadoline. This agent shares the neuroprotective but not the psychomimetic properties of dizocilpine, and is thought to inhibit presynaptic glutamate release.

Male Wistar rats (250g) were given neuroprotective doses of enadoline (1mg/kg), dizocilpine (3mg/kg), or vehicle i.p.. IEG expression was examined 1hr later in 10mm brain sections by *in situ* hybridization using [35 S]-oligonucleotide probes complementary to c-*fos*, c-*jun*, NGFI-A, and NGFI-B. The results were measured as arbitrary optical density units by image analysis. The results for c-*fos* m RNA are summarised in the Table, and simliar results were obtained with the other IEGs measured. Cellular resolution of mRNA distribution was achieved by dipping sections in emulsion. The Table shows that both drugs caused increases in IEGs in dorsal medial thalamic nuclei. In additon, dizocilpine stimulated IEG expression in the cingulate and retrosplenial cortex. Neither drug stimulated expression in the hippocampus.

TABLE : RATIOS OF c-fos mRNA EXPRESSION RELATIVE TO THE HIPPOCAMPUS.[a]

BRAIN AREA	DIZOCILPINE[b]	ENADOLINE[c]
Cingulate cortex	4.76±0.24	0.81±0.08
Retrosplenial cortex	4.61±0.26	0.86±0.17
Paraventricular thalamic nuclei	5.51±0.80	7.80±0.41
Centromedian thalamic nuclei	7.48±0.07	5.11±0.29
Hippocampus	1.0	1.0

a. The results represent the findings from 3 sections from 3 animals in each treatment group. The hippocampal relative optical density units were for dizocilpine mean ± S.E. = 0.011±0.001; enadoline = 0.010±0.001. Kruskal-Wallis non-parametric ANOVA : (b) P = 0.0036; (c) P = 0.024.

The results demonstrate increased expression with both drugs in thalamic nuclei and suggest that increases in IEG expression may be a reflection of changes in excitatory amino acids.

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17P IMMUNONEUTRALIZATION OF LIPOCORTIN 1 REVERSES THE INHIBITORY ACTIONS OF DEXAMETHASONE ON HYPOTHALAMO-PITUITARY-ADRENOCORTICAL (HPA) FUNCTION *IN VITRO* AND *IN VIVO*

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Our previous studies have led us to propose that lipocortin 1 (LC1) mediates some aspects of the powerful inhibitory effects of the glucocorticoids on hypothalamo-pituitary-adrenal (HPA) function at the level of both the hypothalamus (Loxley *et al*, 1993) and the anterior pituitary gland (Taylor *et al*, 1993). In the present study we have tested this hypothesis further by examining, *in vitro* and *in vivo*, the effects of an anti-lipocortin 1 antisera on the capacity of dexamethasone to block the HPA responses to interleukin 1ß (IL1-ß).
For *in vitro* studies, groups of hypothalami (n=5-6), removed post-mortem from either intact or chronically adrenalectomized (surgery under pentobarbitone anaesthesia 7-10 days prior to the experiment) adult (≈200g) male Sprague-Dawley rats, were incubated (Loxley *et al*, 1993) with or without IL1-ß (1ng/ml) and/or dexamethasone (0.1µM) in the presence or absence of a monoclonal anti-lipocortin 1 antibody (MAb, Zymed, diluted 1:15000) or a correspondingly diluted isotype matched antibody (anti-spectrin α+ß, Sigma Chem. Co., UK); CRF-41 released into the medium (pg/ml) was determined by radioimmunoassay. In tissue from adrenalectomized rats, IL1ß produced a significant ($P<0.05$) increase in ir-CRF-41 release (32.1±9.1 vs 8.7±1.9 in controls) in the presence of the control Ab. This response was overcome by pre-incubation (90min) of the tisssue with dexamethasone (6.1±0.9) which, alone, had no effect on basal CRF-41 release (9.4±5.2). Inclusion of the anti-lipocortin 1 MAb had no effect on basal CRF-41 release (14.7±2) or on the response to IL1ß (29.1±4.3). It also failed to influence basal peptide release in the presence of dexamethasone (7.3±0.8) but overcame completely the inhibitory actions of the steroid on the response to IL1ß (102.7±50). A similar profile of data was obtained from tissue from intact rats although changes in peptide release were less pronounced.
For *in vivo* studies, intact rats (≈200g) were injected intra-peritoneally (i.p.) on two successive days with either an affinity-purified polyclonal anti-LC1 Ab (1ml/kg) or an equal volume of normal-sheep serum (NSS) which had been subjected to the same purification procedure ; a third group of rats was handled but not injected (serum free controls, SFC). On day 3, rats from each group were given either dexamethasone (100µg/kg, i.p.) or a corresponding volume of saline (1ml/kg, i.p.) and 1h 15min later a submaximal dose of IL1ß (7.5µg/kg, i.p.) or an equal volume (1ml/kg, i.p.) of its saline vehicle. After a further 60min the rats were killed (decapitation) and the serum collected for corticosterone estimation (ng/ml, by radioimmunoassay). In rats treated with the control NSS, IL1ß caused a significant ($P<0.001$) increase in serum corticosterone (455±106 vs 28±11); this response was overcome by pretreatment with dexamethasone (7±1) which alone significantly ($P<0.01$) reduced the basal ir-corticosterone concentrations (6±0.1). Similar data were obtained in the SFC-group. The anti-LC1 Ab had no effect on the resting ir-corticosterone concentration (13±5) or on the response to IL1ß (785±270). It also failed to influence the resting ir-corticosterone in dexamethasone-treated rats (8±3) but effectively reversed the inhibitory effect of the steroid on the response to IL1ß (450±106 vs 4±2).
The results support our hypothesis that LC1 plays an important role in the feedback regulation of HPA function by glucocorticoids.

We are grateful to The Wellcome Trust and ONO Pharmaceuticals Co., Japan for financial support, to Dr. B. Williams (Edinburgh) for the corticosterone antibody, to Dr. J. Croxtall (London) for the LC1-Ab and to Dr. R.C. Newton, Dupont-Merck, USA and WHO for the cytokines.

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18P MECHANISMS OF EOSINOPHIL ACCUMULATION INDUCED BY INTERLEUKIN-1 IN RAT SKIN: EVIDENCE FOR THE INVOLVEMENT OF PAF

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The pro-inflammatory cytokine interleukin-1 (IL-1) has recently been implicated in the process of antigen-induced eosinophil accumulation and bronchial hyperreactivity in guinea-pigs (Watson *et al.*, 1993). In the present study, in order to investigate directly the role of IL-1 in eosinophil accumulation in vivo, we have examined and characterised the ability of recombinant rat IL-1β (rRIL-1, Ciba-Geigy Ltd) in inducing the local accumulation of ^{111}In-eosinophils in rat skin. These studies were carried out using a model based on our previously reported system in the guinea-pig (Faccioli *et al.*, 1991). Briefly, rat eosinophils (≥94% pure) were purified from horse-serum elicited peritoneal exudates using a discontinous percoll gradient. Cell accumulation and oedema formation, in recipient rats, were simultaneously measured by the local accumulation of i.v. administered ^{111}In-eosinophils and ^{125}I-albumin, respectively. Intradermal administration of rRIL-1 elicited a dose-dependent accumulation of ^{111}In-eosinophils in the dose range of 10^{-14}-5×10^{-13}moles/site which was slow in onset, progressively increasing over the 4h period investigated. Responses (over 4h) induced by rRIL-1 were inhibited when co-injected with the IL-1 receptor antagonist (IL-1ra; Eisenberg *et al.*, 1990) or the protein synthesis inhibitor actinomycin D (ActD) (Table 1).

Table 1 Effect of (A) IL-1ra (2.5×10^{-9}moles/site) and (B) ActD (4.9×10^{-9}moles/site) on rRIL-1 (5×10^{-13}moles/site)-induced responses (mean±s.e.mean for n=7 rats, a significant difference from control responses (2-way ANOVA) is denoted by *$p<0.05$).

A. Treatment	^{111}In-eosinophils/ skin site	Oedema (µl plasma/skin site)	B. Treatment	^{111}In-eosinophils/ skin site	Oedema (µl plasma/skin site)
Tyrode	274±21	15.1±0.6	Tyrode	196±27	16.6±1.9
rRIL-1	737±55	25.9±3.6	rRIL-1	574±64	26.3±2.1
rRIL-1+IL-1ra	400±47*	18.4±2.9	rRIL-1+ActD	337±66*	17.7±1.9
IL-1ra	295±35	17.0±2.5	ActD	220±43	13.4±1.7

In animals pretreated with the potent and specific PAF antagonist UK-74505 (0.5mg/kg i.v.; Cooper *et al.*, 1990), the accumulation of ^{111}In-eosinophils, but not the oedema formation, induced by rRIL-1 (5×10^{-13}moles/site) was significantly inhibited (585±110 and 214±75 cells/site over Tyrode levels in control and UK-74505-treated rats respectively, n=6 pairs, $p<0.01$).

These results demonstrate for the first time that IL-1 can elicit an eosinophil accumulation response in vivo that is receptor-mediated, protein synthesis-dependent and partly mediated by the generation of PAF.

Supported by Consellería de Cultura, Educació y Ciencia (Generalitat Valenciana) and The Wellcome Trust
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19P INFLAMMATORY MEDIATORS OF IRRITANT CONTACT DERMATITIS FROM MOUSE EARS

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Interleukin (IL)-1 which induces inflammatory responses in skin is transcribed *in vivo* in mouse epidermis and bioactive material has been measured (Kilgus *et al.*, 1993). PGE_2 has been shown to be a proinflammatory mediator in arachidonic acid (AA) treated mouse ears (Chang *et al.*, 1986). The aim of this investigation was to induce irritant contact dermatitis (ICD) with AA or calcium ionophore (A23187) and to measure the IL-1 and PGE_2 present in inflamed mouse ears.

Female mice (NMRI and C57BL/6 strains) were treated topically with either 2mg AA (in acetone) or 15µg A23187 (in DMSO/acetone; 10/90) on the right ears whilst the vehicle was applied to the left ears (Chang *et al.*, 1986). The A23187 treated mice were sacrificed at 7.5h and AA treated mice at 90min; these were the times of maximal oedema. Immediately after weighing, the ears were immersed in liquid nitrogen and prepared for assay of biologically active IL-1 and PGE_2 as described (Moore & Cunningham, 1992).

The oedematous response to AA was smaller in the C57BL/6 mice (Table: values represent oedema and mediator levels in the right ear after subtraction of the left ear measurements). In mice treated with vehicle on both ears basal PGE_2 was found to be: NMRI (n=5): left 4.2 ± 0.5, right 3.7 ± 0.4ng/ear, C57BL/6 (n=5): left 7.3 ± 0.4, right 7.2 ± 0.5ng/ear. Topical AA increased the PGE_2 release in the right ear, which was not significant in the C57BL/6 mice. IL-1 was also significantly increased in AA treated ears. Topical A23187 increased oedema similarly in both mouse strains. However, PGE_2 was increased in both ears by approximately 16 times the basal levels (NMRI: right 74.9 ± 6.2ng/ear, left 65.8 ± 5.2ng/ear; C57BL/6: right 94.5 ± 10.7ng/ear, left 97.0 ± 10.7ng/ear) but no increase was seen in IL-1.

	AA induced ICD		A23187 induced ICD	
Mouse strain	NMRI	C57BL/6	NMRI	C57BL/6
Increase in ear weight (mg)	19.83 ± 0.96** (n=36)	11.06 ± 0.65** (n=35)	20.53 ± 1.40** (n=15)	25.87 ± 0.94** (n=15)
Increase in PGE_2 (ng/ear)	6.94 ± 1.26** (n=10)	1.39 ± 1.68 (n=9)	9.05 ± 4.90 (n=10)	-2.5 ± 5.69 (n=10)
Increase in IL-1 (pg†/ear)	553.0 ± 126.6** (n=5)	377.4 ± 131.8* (n=5)	-79.88 ± 89.07 (n=5)	64.26 ± 79.01 (n=5)

† IL-1 expressed as pg human recombinant IL-1 equivalents/ear. **p<0.01; *p<0.05 comparing the right ear to the vehicle treated left ear using Student paired 't'-test.

These results demonstrate that, in agreement with previous studies, PGE_2 levels are lower in mice with smaller oedematous responses to AA and suggest that IL-1 may play a role in this response. The increase in PGE_2 in both ears of A23187 treated mice suggests, not only a systemic effect of the irritant on PG synthesis, but also that an increase in PGE_2 alone is not sufficient to elicit an oedematous response to A23187 induced ICD.

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20P HISTAMINE CO-OPERATES WITH INTERLEUKIN-8 TO INDUCE NEUTROPHIL ACCUMULATION INTO THE MOUSE AIR-POUCH

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Interleukin-8 (IL-8) has been characterised as a specific and direct neutrophil activator (Baggiolini *et al.*,1989). This chemotactic cytokine, with C5a and leukotriene B_4, is considered to be a direct acting chemoattractant as it does not require continuing DNA-dependent RNA synthesis (Colditz *et al.*, 1989). The following study uses the murine air-pouch model to investigate the potential role of histamine in polymorphonuclear leucocyte (PMN) recruitment induced by IL-8.

Air-pouches were formed on the back of male Swiss Albino mice (28-30 g, Tuck, UK) by s.c. injection of 2.5 ml of air on day 0 and 3. Human recombinant IL-8 (72aa, a generous gift of Dr I Lindley, Sandoz, Wien, Austria) in 0.5 ml of carboxymethylcellulose 0.5 % in sterile phosphate buffered solution (PBS) was administered locally at time 0, 6 days after the initial injection of air. Mice were killed 4 h later and the pouches were washed with PBS (+ heparin 50 U ml^{-1}). PMN were counted after staining with Turk's solution (1:10 dilution). In some experiments mice were treated with mepyramine (1-10 mg/kg) by i.p. injection, 15-20 min before local administration of IL-8. In other experiments IL-8 (0.3 µg) and histamine (0.2 to 4 µg) were co-administered into the air pouch at time 0 and leucocyte accumulation was measured 4h later. IL-8 (1 µg)-induced PMN migration was also assessed in mice depleted of mast cell amines by sub-chronic treatment with compound 48/80 (six doses at 0.6 mg/kg i.p at 12 h intervals followed by three doses of the same agent at 1.2 mg/kg). Statistical comparisons were made between the numbers of PMN (10^6 per mouse) recovered by ANOVA followed by Bonferroni test for inter-group comparisons.

Local IL-8 induced a dose-dependent (0.03 to 3 µg) PMN infiltration into the air-pouch measured at the 4h time point with optimal infiltration at IL-8 1µg (CMC: 1.56 ± 0.18 x10^6 PMN, n=47; CMC+ IL-8 1µg: 5.84 ± 0.28 x10^6 PMN, n=70, $p < 0.01$). The selective H_1 antagonist, mepyramine, significantly reduced the PMN infiltration (40-60 % inhibition) induced by IL-8 (1 µg) in a dose-dependent (1-10 mg/kg) manner eg IL-8 1 µg: 6.07 ± 0.46 x10^6 PMN, n=9; IL-8 1 µg + mepyramine 6 mg/kg: 3.20 ± 0.32 x10^6 PMN, n=12, $p < 0.01$. Co-injection of IL-8 (0.3 µg) and histamine (0.2 to 4 µg) potentiated the PMN response, with an optimal histamine dose of 2 µg (IL-8 0.3 µg: 3.28 ± 0.27 x10^6 PMN, n=15; IL-8 0.3 µg + histamine 2 µg: 4.34 ± 0.30 x10^6 PMN, n=14, $p < 0.05$). Impaired PMN infiltration by IL-8 was observed in mice depleted of mast cell amines because of subchronic treatment with compound 48/80 (IL-8 1 µg: 10.3 ± 1.15 x10^6 PMN, n=6; compound 48/80 + IL-8 1 µg: 5.8 ± 0.54 x10^6 PMN, n=7, $p < 0.01$).

In conclusion, the results support the concept that histamine and IL-8 co-operate to recruit PMN into the air-pouch. These mediators may also possibly interact in more complex inflammatory situations to induce PMN infiltration.

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The financial support of Ono Pharmaceutical Co. Japan is acknowledged. RJF is funded by an endowment from Lilly.

21P MECHANISMS OF IMMUNE COMPLEX-INDUCED INFLAMMATION IN GUINEA-PIG SKIN

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The reversed passive Arthus (RPA) reaction is an acute, immune complex-mediated inflammatory reaction characterized by haemorrhage, oedema formation, localized platelet deposition and neutrophil accumulation. In the RPA reaction in rabbit skin, both oedema formation and haemorrhage are dependent on neutrophils as assessed by the capacity of an anti-CD18 antibody to inhibit these features of the reaction (Norman et al, 1993). In contrast, local oedema formation in guinea pig skin appears to be independent of neutrophil accumulation (Teixeira et al, 1993). In this study, we investigated the mechanisms underlying the RPA reaction in guinea pig skin and tested whether an anti-CD18 antibody, by inhibiting neutrophil accumulation, could interfere with local oedema formation and haemorrhage in this reaction. Casein-elicited neutrophils were purified on a discontinuous Percoll gradient and radiolabelled with $^{111}InCl_3$. Red blood cells (RBC) were obtained by cardiac puncture and radiolabelled with $Na^{51}Cr$. The ^{111}In-neutrophils ($5x10^6$), ^{51}Cr-RBC (0.25 ml of blood) and ^{125}I-human serum albumin (~5 μCi) were injected i.v. into recipient guinea pigs. Five min later, these animals received i.d. injections of inflammatory stimuli or increasing concentrations (6.25 to 100% in saline) of a rabbit anti-bovine gamma globulin (BGG) anti-serum followed immediately by i.v. injection of antigen (BGG, 5 mg/kg). The guinea pigs were sacrificed after 4 hours. For the local treatments, drugs were mixed with inflammatory stimuli or anti-serum just before i.d. injections. The anti-CD18 antibody (6.5E - $F(ab')_2$) was injected i.v. 10 minutes prior to i.v. injections of radiolabelled cells and albumin. Results are expressed as mean ± s.e. mean.

In the RPA reaction, there was a dose- and time-dependent accumulation of ^{111}In-neutrophils and oedema formation. Whereas oedema formation was complete by 1 hour, ^{111}In-neutrophil accumulation was still rising at 4 hours. The co-injection of mepyramine ($2.5x10^{-7}$moles/site) with anti-BGG inhibited oedema formation (e.g. RPA 25%, 83.4 ± 2.6 μl; RPA + mepyramine, 45.1 ± 1.9 μl; saline, 17.8 ± 1.4 μl; $P < 0.01$, n= 4). HOE 140 (a bradykinin type 2 receptor antagonist) had no effect on oedema formation in the RPA reaction. Similarly, the PAF antagonist, WEB 2086 (10^{-7}moles/site), or the 5-lipoxygenase inhibitor, ZM 230487 (10^{-8}moles/site) (Crawley *et al*, 1993), had no effect on oedema formation or ^{111}In-neutrophil accumulation in the RPA reaction (e.g. oedema formation: RPA 25%, 105.9 ± 9.2 μl; RPA + WEB, 102.5 ± 18.2 μl; RPA + ZM, 86.8 ± 16.5 μl; ^{111}In-neutrophil accumulation: RPA 25%, 3126 ± 294; RPA + WEB, 3096 ± 469; RPA + ZM, 2860 ± 196; n= 4). Systemic pre-treatment with 6.5E (2.4 mg/kg) effectively inhibited neutrophil accumulation in RPA sites by 93%. Oedema formation measured in the same sites was unaltered whereas haemorrhage (^{51}Cr-RBC extravasation) was completely inhibited at lower doses of anti-serum (e.g. RPA 50%, 2.10 ± 0.16 μl of blood; RPA + 6.5E, 1.22 ± 0.24 μl; saline, 1.34 ± 0.08 μl; $P < 0.05$, n= 6). Thus, in immune complex-mediated inflammation in guinea pig skin, haemorrhage, but not oedema formation, seems to depend on neutrophil function. A significant part of oedema formation was due to endogenous histamine. We found no evidence for the participation of bradykinin, PAF or leukotrienes in mediating either oedema formation or neutrophil accumulation in this model.

We are grateful to the National Asthma Campaign, British Lung Foundation, Wellcome Trust and Sandoz, Basle for support.

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22P ROLE OF THE CD11b/CD18 RECEPTOR AND PLATELET ACTIVATING FACTOR IN THE REGULATION OF INTERLEUKIN-8 RELEASE FROM ZYMOSAN-STIMULATED HUMAN NEUTROPHILS

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The neutrophil chemoattractant, interleukin-8 (IL-8), has been detected in inflammatory exudates induced by i.p. injection of zymosan (ZYM) in the rabbit (Beaubien *et al*, 1990; Collins *et al*, 1991). In-vitro, ZYM-stimulated neutrophils have been shown to release IL-8 (Bazzoni *et al*, 1991) however the underlying mechanisms have not been determined. We have investigated the regulation of IL-8 production by human neutrophils as such mechanisms could be of considerable importance in controlling cell recruitment into inflammatory sites in-vivo. Purified human neutrophils (>98%) were resuspended in RPMI 1640 medium + 10% FCS. Cells ($2x10^6$/well) were incubated with ZYM ($1x10^7$ particles/well) for 24h in tissue culture plates (500μl/well). Polymixin B sulphate (20μg/well) was routinely added to inhibit the action of any contaminating LPS. IL-8 levels in the supernatant were determined by radioimmunoassay. The effect of two PAF antagonists (WEB 2086 and UK 74505), two anti-CD18 (60.3, and 6.5E) antibodies and an anti-CD11b (KIM 225) antibody, on ZYM-induced IL-8 release was investigated by addition of the drugs together with, or at various times after, the ZYM stimulus.

Table 1. Effect of anti-CD18 (60.3, 6.5E) and anti-CD11b (KIM 225) antibodies on ZYM-induced IL-8 generation from human neutrophils

			+MoAb 60.3(μg/well)			+MoAb 6.5E(μg/well)				+MoAb KIM 225(μg/well)				
	Unstim	ZYM	0.3	1.0	3.0	ZYM	0.3	1.0	3.0	ZYM	0.3	1.0	3.0	10.0
IL-8(nM)	0.15	3.70	2.11	0.78*	0.67*	2.26	1.69	0.42*	0.16*	2.26	2.28	1.67	1.40*	0.87*
(n=4-5)	±0.05	±0.5	±0.3	±0.3	±0.2	±0.4	±0.7	±0.2	±0.1	±0.4	±0.4	±0.4	±0.3	±0.2

Both of the anti-CD18 antibodies and the anti-CD11b antibody inhibited ZYM-induced IL-8 generation in a dose-dependent fashion (table 1; *$p<0.01$). Isotype-matched control antibodies (MOPC 21 for 6.5E and KIM 225; 1.24 for 60.3) had no significant effect on zymosan-induced IL-8 levels at 24 hours. In time course studies MoAb 60.3 (anti-CD18; 3μg/well) produced maximal inhibitory effects if co-administered with the ZYM stimulus, or added within 1 hour of stimulation. No inhibition was observed if addition was delayed by 3h. Similarly, the PAF receptor antagonists WEB 2086 (10^{-6}M) and UK 74,505 (10^{-8}M) inhibited ZYM-induced IL-8 production from neutrophils at 24 hours (69% and 74% reduction respectively compared with the response to ZYM alone, $p<0.01$). As observed with the anti-CD18 antibodies, maximal inhibitory effects were achieved with co-administration (or addition within 30 min) of the antagonists with ZYM. No significant inhibition was seen if the addition of the antagonists was delayed by more than 1 hour. These results suggest that ZYM-induced IL-8 release from neutrophils involves "inside signalling" via the CD11b/CD18 receptor. In addition, evidence has been obtained for an autocrine role for endogenously generated PAF, or a PAF-like molecule, in regulating the response.

This work was supported by Fisons UK, The Wellcome Trust & The National Asthma Campaign.

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23P PRO- AND ANTI-INFLAMMATORY EFFECTS OF THE STABLE PGD_2 ANALOGUE, ZK 118.182

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In the rabbit, PGI_2 and 15-methyl-PGE_1 can be either pro- or anti-inflammatory, depending on their route of administration (Rampart & Williams, 1986). Here we have investigated the effects of local and systemic administration of the stable PGD_2 analogue, ZK 118.182 (Schultz et al., 1990) on inflammatory responses in rabbit skin. Furthermore, we have studied the possible mechanisms underlying the anti-inflammatory effects of the PGD_2 analogue by performing *in vitro* experiments. Oedema formation and neutrophil accumulation were quantified in the skin of anaesthetized rabbits. The accumulation of ^{125}I-albumin and ^{111}In-neutrophils were measured at skin sites 30 min after i.d. injections of neutrophil-dependent stimuli, such as FMLP and leukotrieneB_4 (LTB_4) or agents that act directly on endothelial cells to cause increased microvascular permeability, such as bradykinin (Bk) and PAF. *In vitro*, superoxide anion (O_2^-) production by purified peripheral neutrophils was assessed by a colorimetric reaction measuring the superoxide-inhibitable reduction of cytochrome C and neutrophil adherence determined by measuring the adherence of ^{111}In-labelled neutrophils to gelatin-coated microtitre plates. All results are expressed as the mean ± s.e.mean.

ZK 118.182, like PGD_2 and PGE_2, when co-injected with Bk into rabbit skin, induced a dose-dependent increase in plasma leakage. For example, i.d. injected saline, Bk (10^{-10} mol/site), ZK 118.182 (3 x 10^{-9} mol/site) and Bk plus ZK 118.182 induced 4.0±0.5, 23.8±5.4, 6.2±0.5 and 51.9±10.2 µl plasma/site respectively (n=6). In animals infused i.v. with saline, oedema formation induced by FMLP ($5x10^{-11}$ mol/site), LTB_4 ($5x10^{-10}$ mol/site), PAF (10^{-9} mol/site) and Bk (10^{-10} mol/site), all in the presence of local PGE_2 (3 x 10^{-10} mol/site), were 40.2±5.4, 36.6±2.5, 47.9±4.0, 71.3±6.1 µl/plasma respectively. In rabbits infused i.v. with ZK 118.182 (0.45 µg kg^{-1} min^{-1}) oedema formation induced by FMLP (24.9±1.5 µl/plasma; $P<0.01$; n=16) and LTB_4 (25.6±1.8 µl/plasma; $P<0.05$,; n=14) were significantly inhibited, whereas responses to PAF (37.2±5.6 µl/plasma; n=7) and Bk (58.5±5.1 µl/plasma; n=18) were not affected. Accumulation of neutrophils induced by LTB_4 and FMLP, both in the presence of PGE_2, was not affected by systemic ZK 118.182 (n=8). *In vitro*, FMLP at 10^{-7}M and 10^{-9}M caused 4.6±0.4 and 3.6±0.4 nmol/$5x10^5$ cells O_2^- production respectively. These responses were reduced to 3.5±0.3 ($P<0.01$) and 1.5±0.2 ($P<0.001$) in the presence of 10^{-6} M ZK 118.182 and to 2.8±0.2 ($P<0.01$) and 1.3±0.1 ($P<0.001$) with 10^{-5}M ZK 118.182 (n=4-6). Only adherence induced by 10^{-7}M FMLP (42.3±4.7% of total adherence) was reduced by 10^{-5}M ZK 118.182 (26.9±6.4%; $P<0.05$; n=6); 10^{-6}M ZK 118.182 had no significant effect on FMLP-induced adherence (n=6).

The results show that, depending on the route of administration, the PGD_2 analogue can have either pro- or anti-inflammatory effects. The selective anti-inflammatory effect of ZK 118.182 towards neutrophil-dependent mediators may be related to its ability to inhibit increased microvascular permeability induced by neutrophils, without interfering with neutrophil accumulation. This latter effect may be due to the ability of the compound to suppress neutrophil secretion to a greater extent than neutrophil adherence.

This work was supported by the Wellcome Trust, Schering AG and the National Asthma Campaign.
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24P LIPOCORTIN 1 LEVELS IN LUNG LAVAGE FLUIDS COLLECTED FROM HEALTHY VOLUNTEERS BEFORE AND AFTER TAKING EXOGENOUS GLUCOCORTICOIDS

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Some of the anti-inflammatory actions of the glucocorticoids are likely to be mediated by lipocortin 1 (Lc1; Flower, 1988), a protein found in many organs including the lung, where it may originate, in part, from Type II epithelial cells (Smith et al, 1990). In this study, we have assessed the glucocorticoid-inducibility of Lc1 in healthy human lungs.

After giving informed consent, on study day 1, 7 asymptomatic male volunteers, aged 24 - 35 yr, median 28 yr, underwent bronchoalveolar lavage (BAL-1) of the right middle lobe (RML) with 4 x 50 ml sterile, 0.15M NaCl between 8.30 am and 10 am. On days 2- 8 inclusive each took 30mg prednisolone orally between 6.30 am and 7 am. On day 8, within 3h of taking the drug each underwent a second lavage of the RML (BAL-2) as before and 6 also underwent lavage of the left upper lobe (LUL; BAL-3). BAL fluids (BALF) were processed and stored at -70°C immediately after collection and extracellular levels of total protein, albumin and Lc1 in BALF were quantified (Table 1) as described before (Smith et al, 1985; 1991).

Table 1: TOTAL PROTEIN, ALBUMIN (ALB) AND Lc1 IN BALF BEFORE AND AFTER PREDNISOLONE

	n	Volume (ml)	Protein (ug/ml)	Albumin (ug/ml)	Lc1 (ng/ml)	Lc1/Alb (pg/ug)
BALF-1	7	115(90-160)	130(43-176)	30.5(12.3-72.8)	3.4(1.2- 6.3)	98(48-350)
BALF-2	7	135(77-163)	112(77-280)	31.3(7.0-35.5)	3.6(0.6- 9.2)	128(17-346)
BALF-3	6	99(85-118)[a]	134(102-208)	35.0(16.5-50.0)	6.9(0.9-12.2)[b]	253(19-414)[c]

Median (Range); [a]<BALF-1, [b]>BALF-2, [c]>BALF-1, $P<0.05$, Wilcoxon test for paired data.

After prednisolone, Lc1 was significantly higher in BALF from the LUL (BALF-3) than from the RML before (BALF-1; P=0.047) or after (BALF-2; P=0.047) treatment. Although Lc1 levels may be intrinsically higher in the LUL, the RML may be depleted of Lc1 by the first lavage. If so, then prednisolone may stimulate replenishment, but not significant enhancement of the Lc1 in this lobe.

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25P CHARACTERISATION OF THE PGE RECEPTOR SUBTYPE MEDIATING INHIBITION OF SUPEROXIDE PRODUCTION IN HUMAN NEUTROPHILS

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Superoxide generation by activated neutrophils is inhibited by PGE_2 through activation of EP receptors (Wheeldon & Vardey, 1993). The aim of the present study was to characterise the EP receptor subtype involved and to test the hypothesis that cyclic AMP is the second messenger mediating inhibition of neutrophil activation. For these purposes, the inhibitory effects of selective EP agonists have been evaluated. Also the modulatory effects of the adenylate cyclase inhibitor, SQ 22,536 (Harris et al., 1979) and the phosphodiesterase inhibitor, IBMX have been assessed.

Fresh heparinised human blood was layered onto mono-poly resolving medium and centrifuged at 650 g for 30 min at 20°C, to isolate the neutrophil fraction. Neutrophils (1.5 x 10^6 cells/ml) were preincubated in PBS (37°C) for 10 min, with prostanoids or vehicle in presence of cytochalasin B (5µg/l) and cytochrome C (1mg/ml) prior to stimulation with FMLP (100nM) for 10 min. Superoxide generation was assayed by spectrophotometric evaluation of the reduction of cytochrome C (A550nm).

PGE_2 caused concentration-related inhibition of FMLP-induced superoxide generation (Table 1) EC_{50} = 0.18 ± 0.04 µM, n=10. IBMX (0.25 mM) shifted the concentration-effect curves to PGE_2 (n=13) to the left (Table 1). Further experiments carried out in the presence of IBMX showed that the selective EP_2-agonists, 11-deoxy PGE_1, butaprost and AH 13205, also caused concentration-related inhibition of FMLP-induced superoxide generation (Table 1) with equieffective concentration ratios (EEC, PGE_2=1) of 5.3, 12.7 and 50.7, respectively. In contrast, sulprostone (EP_1/EP_3 agonist) and 17-phenyl PGE_2 (EP_1 agonist) showed no effect till 10 µM, giving EECs of >700 and 486 respectively (n=3). The involment of cyclic AMP is suggested by the findings that, SQ 22,536 (100 µM, 2 min) significantly ($p<0.05$) attenuated inhibition of superoxide by PGE_2 (EC_{50}s of 0.24 ± 0.1 and 1.9 ± 1.1 µM) and that IBMX itself reduced the FMLP response by 54.9 ± 5.0%.

Table 1 Percentage inhibition of the superoxide generation in human neutrophils (mean ± s.e.mean, n=4)

Concentration (µM)	0.001	0.01	0.1	1.0	10.0
PGE_2	4.1 ± 1.1	11.8 ± 2.9	29.7 ± 5.3	48.1 ± 6.0	62.6 ± 6.6
PGE_2 + IBMX	24.0 ± 6.6	52.3 ± 6.4	65.3 ± 6.5	73.3 ± 5.3	83.9 ± 5.2
11-deoxy PGE_1 + IBMX	6.9 ± 3.3	30.6 ± 6.1	47.9 ±3.0	68.9± 3.3	88.7 ± 5.8
butaprost + IBMX	14.7 ± 9.5	15.2 ± 7.4	41.4 ±3.5	53.7± 4.2	61.3 ± 6.2
AH 13205 + IBMX	2.5 ± 0.9	5.7 ± 5.7	31.1 ±8.9	54.4± 2.9	67.2 ± 2.2

The results of the present study suggest that inhibition of superoxide generation by prostanoids is mediated by occupation of EP_2 receptors and activation of adenylate cyclase, leading to the elevation of intracellular levels of cyclic AMP.

Elodie Talpain is funded by a Glaxo PhD studentship

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26P CYCLIC NUCLEOTIDE PHOSPHODIESTERASES IN HUMAN EOSINOHILS: FUNCTIONAL AND BIOCHEMICAL EFFECTS OF ISOENZYME SELECTIVE INHIBITORS

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Guinea-pig peritoneal eosinophils express a membrane-associated cyclic AMP hydrolysing activity which has the characteristics of a type IV phosphodiesterase (PDE) isoenzyme. Moreover, selective inhibitors of the PDE IV isoenzyme family effectively suppress respiratory burst activity by these cells (Dent *et al.*, 1991; Souness *et al.*, 1991). In this abstract, we report the identification and partial characterisation of the PDE isoenzymes in *human* eosinophils, the effect of PDE inhibitors and a β-adrenoceptor agonist upon serum-opsonised zymosan (SOZ)-induced respiratory burst, and the interaction between salbutamol and the PDE inhibitors rolipram and zardaverine upon cyclic AMP accumulation and superoxide anion ($\cdot O_2^-$) generation.

Human eosinophils were purified from the peripheral blood of atopic and eosinophilic volunteers by the negative selection immunomagnetic separation technique of Hansel *et al.*, (1991). Cyclic AMP mass, PDE activity and $\cdot O_2^-$ generation were measured as described previously (Dent *et al.*, 1991).

Human eosinophil lysates preferentially hydrolysed cyclic AMP over cyclic GMP (1.73 ± 0.37 *vs* 0.09 ± 0.03 pmol/min/10^6 cell eq respectively at 1 uM substrate, n = 8). Cyclic AMP hydrolysis was not significantly affected by cyclic GMP (10 µM) or by calmodulin (25 U) and Ca^{2+} (1.5 mM), but was potently suppressed by representative inhibitors of the PDE III/IV (zardaverine: IC_{50} = 588.2 ± 148.4 nM, n = 5), PDE IV (rolipram: IC_{50} = 568.4 ± 97.7 nM, n = 4; Ro 20,1724 = 3.1 ± 0.47 µM, n = 5) and PDE IV/V (denbufylline: IC_{50} = 361.1 ± 19.1 nM, n = 3) isoenzyme families. In contrast, the PDE III inhibitor SK&F 94120 was inactive (IC_{50} > 100 uM). Kinetic analysis of cyclic AMP hydrolysis by the method of Hofstee showed non-linear kinetics that could be resolved into high affinity (K_{mapp}: 9.81 µM) and low affinity (K_{mapp} = 601.1 µM) components. In intact eosinophils, SOZ (1 mg/ml) promoted the generation of $\cdot O_2^-$ that was inhibited in a concentration-dependent manner by rolipram (IC_{50} = 48.9 µM (2.4 µM - 1 mM 95% C.L.), n = 4) zardaverine (IC_{50} = 29.7 µM (2.6 - 344 µM), n = 10) and salbutamol (IC_{50} = 20.4 µM (38 nM - 1.1 mM), n = 3). Curiously, both zardaverine and rolipram interacted with salbutamol in a purely additive manner; no synergy was observed. In contrast, rolipram and zardaverine potentiated the ability of salbutamol to increase the cyclic AMP content (**Rolipram**: control = 0.25 ± 0.025, rolipram (100 µM) = 0.66 ± 0.15, salbutamol (10 µM) = 0.37 ± 0.06, rolipram + salbutamol = 2.99 ± 0.68 pmol/10^6 cells, n = 4; **Zardaverine**: control = 0.23 ± 0.03, zardaverine (100 µM) = 0.86 ± 0.19, salbutamol = 0.38 ± 0.04, zardaverine + salbutamol = 2.29 ± 0.5 pmol/10^6 cells, n = 4).

It is concluded that human eosinophils express a PDE IV isoenzyme that is responsible for regulating the intracellular cyclic AMP content and the ability of these cells to generate $\cdot O_2^-$. The failure of rolipram and zardaverine to potentiate the inhibition of $\cdot O_2^-$ generation induced by salbutamol suggests that a cyclic AMP-*independent* mechanism may underlie the suppressive effect of β-adrenoceptor agonists on human eosinophil oxidative metabolism.

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27P CARDIAC HAEMODYNAMIC EFFECTS OF CHRONIC LIPOPOLYSACCHARIDE (LPS) INFUSION IN CONSCIOUS RATS

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Recently, we described the regional haemodynamic changes accompanying 24 h infusion of LPS (E.Coli serotype 0111:B4 (Sigma); 150 μg kg^{-1} h^{-1}) in conscious Long Evans rats (Gardiner *et al.*, 1992). From those experiments it appeared that changes in cardiac haemodynamics might have been occurring, so we have performed the same experiment in 12 conscious Long Evans rats, chronically instrumented with intravascular catheters and an electromagnetic flow probe on the ascending aorta to assess changes in cardiac function. All surgery was carried out under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p.). Baseline values for heart rate (HR), mean arterial blood pressure (MAP), cardiac index (CI), peak aortic flow (PF), maximum rate of rise of aortic flow (dF/dt_{max}), stroke index (SI), total peripheral conductance (TPC) and central venous pressure (CVP) are shown in Table 1, together with the changes during LPS infusion.

Table 1. Baseline cardiovascular variables and changes during LPS infusion. Values are mean ± s.e. mean (n = 12)

		Changes during LPS infusion			
	Baseline	**1 h**	**3 h**	**8 h**	**24 h**
HR (beats min^{-1})	377 ± 6	18 ± 10	-20 ± 6*	66 ± 7*	59 ± 11*
MAP (mm Hg)	103 ± 2	-7 ± 3	2 ± 2	-7 ± 4	-24 ± 2*
CI (ml min^{-1} 100 g^{-1})	26.1 ± 0.6	-5.2 ± 0.8*	-3.7 ± 0.8*	7.9 ± 0.8*	15.2 ± 1.2*
PF (ml min^{-1} 100 g^{-1})	106 ± 3	-7 ± 2*	-9 ± 2*	10 ± 2*	24 ± 3*
dF/dt_{max} (l min^{-2} 100 g^{-1})	450 ± 14	-21 ± 16	-62 ± 12*	32 ± 12	130 ± 16*
SI (μl $beat^{-1}$ 100 g^{-1})	70 ± 2	-16 ± 2*	-7 ± 3	7 ± 1*	25 ± 2*
TPC (μl min^{-1} mm Hg^{-1} 100 g^{-1})	254 ± 9	-37 ± 12*	-39 ± 9*	102 ± 16*	266 ± 19*
CVP (cm H_2O)	3.5 ± 0.3	0.2 ± 0.3	-0.7 ± 0.2*	-0.4 ± 0.3	0.5 ± 0.4

* P<0.05 versus baseline (Friedman's test)

Although after infusion of LPS for 1 h there were no significant changes in MAP or HR, there were reductions in CI, PF, SI and TPC. Thereafter, all cardiac variables increased, such that, by 24 h after onset of LPS infusion, a significant hypotension was accompanied by elevations in HR, CI, PF, dF/dt_{max} and SI, and there was a significant vasodilatation (Table 1). Hence, in this model of endotoxaemia, depression of cardiac function does not appear to contribute to the hypotension.

This work was supported by the British Heart Foundation.

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28P BENEFICIAL EFFECT OF HIRULOG IN RAT ENDOTOXIN SHOCK

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Hirulog is a twenty amino acid peptide with two domains: a carboxy-terminal domain, derived from that of hirudin, which binds to both fibrinogen recognition site of thrombin and platelet membrane receptor, and a catalytic site inhibitor domain. Hirulog is a potent anticoagulant and it has been also shown to prevent generation of inflammatory mediators from endothelial cells in vitro (Prescott et al., 1990) . Here we have tested the ability of hirulog to interfere with some manifestations of endotoxin shock. Male wistar rats (200-250 g) were anaesthetized with urethane (10% w/v; 1 g kg^{-1}, i.p.). and the left jugular vein and the right carotid artery were cannulated. Blood pressure changes were mesaured through the carotid artery by mean of a pressure transducer Beckmann type 9872 connected to a Beckmann polygraph type R511A. Lipopolysaccharide from E.coli (LPS type 0127:B8; Sigma) was injected through the vein at a dose of 25 mg kg^{-1}. Hirulog 1-10 mg kg^{-1} or an equal volume of saline was administered i.v. 30 min prior to LPS administration. Changes in blood pressure were expressed as the total area under the curve calculated by Simpson 's rule. In rats treated with saline the area of hypotension was 302±31 (n=10) while for hirulog at the dose of 1-5 and 10 mg kg^{-1} i.v. was respectively 238±58 (n=6; ns), 120±39 (n=6; $p < 0.05$) and 149±26 (n=6; $p < 0.05$). In another set of experiments hirulog (1-10 mg kg^{-1} i.v.) was given before LPS and the survival rate was evaluated after 3, 6, 12, 24 hours. Survival rate after 24 hours was 15% for the control while at the dose of 5 and 10 mg kg^{-1} i.v. of hirulog was 54% (n=11; $p < 0.05$ Fisher 's test) and 71% (n=7; $p < 0.05$; Fisher 's test), respectively. Furthermore , platelet and leucocyte count was assessed immediately before the LPS administration and 40, 60, 90 and 120 min afterwards. LPS-induced reduction in leucocyte and platelet count was significantly inhibited by hirulog at the dose of 10 mg kg^{-1} i.v. Indeed, leucocyte count at time 0 was 12±0.98 x 10^6 cells ml^{-1} (n=14) and was significantly reduced to 8.3±0.6 x 10^6 and 6.8±1.1 x 10^6 cells ml^{-1}, after 40 and 60 min from LPS administration , respectively. Hirulog at the dose of 10 mg kg^{-1} was able to prevent the fall in leucocyte count. Indeed, leucocyte count after 40 min and 60 min was 12.5±3.4 x 10^6 cell ml^{-1}(n=6; ns) and 12.4±0.8 x 10^6 cell ml^{-1} (n=6; ns). A simliar pattern of inhibition has been observed on change in platelet count 40 min after LPS administration. Prior to LPS administration platelet count was 727±28 x 10^6 ml^{-1} (n=14). After 40 min was 540±72 x 10^6 ml^{-1} (n=14; $p < 0.05$) for control and 691±38 x 10^6 ml^{-1} (n=6; n.s.) for hirulog 10 mg kg^{-1}.
In conclusion our data show that hirulog has beneficial effect in rat endotoxin shock and that this effect is not only related to its anticoagulant activity. It is feasible that hirulog modulates thrombin-induced cellular activation in bacteremia.

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29P MODULATION OF SIGNAL MOLECULE PRODUCTION IN PAF-STIMULATED RABBIT PLATELET BY CALYCULIN A

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Relatively little attention has been focused on the role of protein phosphatases in platelet signalling when compared to the extensive investigations carried out to determine the role of protein kinases. In this study we have investigated the role of type 1 (PP1) and type 2A (PP2A) serine/threonine specific phosphatases in PAF activated platelets using the PP1/PP2A inhibitor calyculin A (Bialojan & Takai, 1988). The effect of calyculin A has been examined on protein phosphorylation in ^{32}P loaded platelets, on PAF-induced inositol-(1,4,5)-trisphosphate production, using a binding assay and on cytosolic calcium ($[Ca^{2+}]_i$) levels using fura-2 loaded platelets (Murphy & Westwick, 1992).

Inhibition of PP1/PP2A activity by pretreatment of platelets with calyculin A (10 - 300 nM) for 10 minutes induced an increase in the basal level of total protein phosphorylation. The same treatment, however, had no effect on the basal level of $[Ca^{2+}]_i$ (69.5 ± 7.3 nM), but caused a dose-dependent inhibition of $[Ca^{2+}]_i$ elevation induced by 3 nM PAF (396 ± 25 nM) with an IC_{50} of 66 ± 4.5 nM calyculin A. Ca^{2+} mobilisation from the intracellular stores (determined by chelation of extracellular Ca^{2+}; $[Ca^{2+}]_e$) was inhibited with a similar IC_{50} of 70 ± 11 nM. Calyculin A was also found to inhibit influx of divalent cations across the plasma membrane, demonstrated as an inhibition in the quench of the fluoresence signal in the presence of Mn^{2+} (Merritt *et al.*1989). Moreover, in PAF-stimulated platelets removal of Ca^{2+} from the cytoplasm was accelerated upon the addition of calyculin A post-PAF either in the presence or absence of $[Ca^{2+}]_e$. Calyculin A (10 - 300 nM) had no effect on the basal level of $Ins(1,4,5)P_3$ which was 10.8 ± 4.8 pmol/10^9 platelets, but caused a dose-dependent inhibition of the production of $Ins(1,4,5)P_3$ measured at 5s post-PAF with an IC_{50} value of 71 ± 15 nM. The effect of calyculin A was also examined on phosphorylation of a 47 kDa protein substrate for protein kinase C. Activation with PAF caused a rapid increase in phosphorylation of the PKC substrate, which reached maximal at 30-60 s post-PAF and then declined to basal levels. However, in platelets pretreated with 300 nM calyculin A, there was a rapid phosphorylation of the PKC substrate, but no dephosphorylation. Incubation of platelets for 20 minutes with the selective PKC inhibitor Ro 31-7549/001 (0.3 - 10 μM) which causes a dose-dependent inhibition of phosphorylation of the 47 kDa substrate of PKC (Murphy & Westwick, 1992) induced a dose-dependent reversal of the inhibitory effect of calyculin A (300 nM) on PAF-induced $[Ca^{2+}]_i$ elevation with an almost complete reversal of the inhibitory effect at 10 μM Ro 31-7549/001, a concentration which inhibits PKC substrate phosphorylation by 95%.

In conclusion, this study has demonstrated that serine/threonine specific protein phosphatases type 1 and type 2A are important in maintaining levels of protein phosphorylation in resting platelets. The finding that calyculin A causes an inhibition in the elevation of platelet signalling molecules in PAF-stimulated platelets, suggests a positive role for PP1/PP2A in PAF-induced platelet signal transduction.

We are grateful to the British Heart Foundation for financial support.

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30P LYSOPHOSPHATIDIC ACID STIMULATES THE TYROSINE PHOSPHORYLATION OF MITOGEN-ACTIVATED PROTEIN KINASE IN THE ENDOTHELIAL CELL LINE EAhy 926

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Lysophosphatidic acid (LPA), is a potent mitogen in fibroblast cell lines in culture. This response is mediated through the activation of multiple intracellular signalling pathways including hydrolysis of $PtdIns(4,5)P_2$ (Plevin *et al.*, 1991) and inhibition of adenylyl cyclase (van Corven *et al.*, 1989). However, little is known of the possible physiological functions of LPA in cells of the cardiovascular system. Recently it has been shown that LPA is released from thrombin-activated platelets (Eichholtz *et al.*, 1993) suggesting a potental site of action in the vessel wall. We have therefore examined the effects of LPA upon tyrosine phosphorylation of mitogen activated protein kinase (MAP) kinase in the endothelial cell line EAHy 926.

All experiments were performed on cells cultured on six well plates and quiesced in serum free conditions for 40 hours. Protein tyrosine phosphorylation was examined by Western blotting using an anti-phosphotyrosine polyclonal antibody. MAP kinase pp42kDa was identified using the anti-MAP kinase monoclonal antibody, MK12. All antibodies were purchased from Affiniti Research Products Ltd, Nottingham, England.

LPA (10μM) stimulated the tyrosine phosphorylation of a 40-45kDa protein which corresponded to the pp42kDa isoform of MAP kinase (density units ± S.D.; control = 0.018± 0.02 LPA 5min = 0.407± 0.17, n=6). The phosphorylation was sustained for at least 60 min although the levels were 39.4%± 24 (n=6) of that obtained after 5 min. The effects of LPA were observed over the nanomaolar concentration range EC_{50} = 187± 14nM, (n=4). The protein kinase C activator 12-O-tetradecanoyl phorbol 13-acetate (TPA) at 100nM also stimulated the sustained tyrosine phosphorylation of MAP kinase. This response was abolished by pretreatment with 30μM of the protein kinase C inhibitor, Ro-318220 (Davies *et al.*, 1989) However, LPA-stimulated phosphorylation was only partly reduced (% inhibition = 63.4%± 12, n=3), and the degree of inhibition was dependent on the time of LPA stimulation (% inhibition; 5min= 73.0%± 12, 60min = 9.5%± 1.7, n=3). Pretreatment with pertussis toxin (18hrs, 100ng/ml) also reduced LPA-stimulated MAP kinase tyrosine phosphorylation in a manner dependent on the time of stimulation (% inhibition 2min = 28%± 12, 60min 73%± 21, n=4). A combination of Ro-318220 and pertussis toxin abolished LPA-stimulated tyrosine phosphorylation of MAP kinase at all time points studied.

These results suggest that, in the endothelial cell line EAhy 926, the regulation of MAP kinase in response to LPA involves both protein kinase C-dependent and -independent pathways with the PKC independent pathway possibly involving the activation of a pertussis toxin sensitive G-protein. In addition these results also suggest a possible role for LPA as an angiogenic factor.

We wish to thank Dr Anne Crilley (Dept. Medical Biochemistry, Glasgow Royal Infirmary) for the gift of the EAhy 926 cell line and Dr G. Lawton (Roche Products Ltd, Welwyn Garden City) for the gift of Ro-318220. This work was funded by the British Heart Foundation.

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31P LYSOPHOSPHATIDIC ACID STIMULATES THE TYROSINE PHOSPHORYLATION OF FOCAL ADHESION KINASE pp125^{FAK} IN RAT-1 FIBROBLASTS

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The phospholipid lysophosphatidic acid (LPA) has been shown to be a potent mitogen in rat fibroblasts. Its effects are believed to be mediated through the activation of a putative extracellular membrane receptor. LPA stimulates the hydrolysis of PtdIns(4,5)P_2 (Plevin *et al.*, 1991) and the inhibition of adenylyl cyclase (van Corven *et al.* 1989), however it is believed that additional pathways are involved in mediating the actions of this phospholipid. We have therefore examined the effects of LPA upon tyrosine phosphorylation in Rat -1 fibroblasts.

In all experiments cells were grown on 6 well plates and quiesced in serum free conditions for 40 h. Protein tyrosine phosphorylation was measured by Western blotting using antiphosphotyrosine antibodies (Affiniti Research Products Ltd, Nottingham). Focal adhesion kinase pp125^{FAK} was immunoprecipitated from lysates of whole cells using the mouse monoclonal antibody 2A7. Protein kinase C activity was measured using the glycogen synthase (G.S.) peptide as a substrate for PKC mediated phosphorylation (Heasley & Johnson, 1989).

LPA (10μM) stimulated the tyrosine phosphorylation of major protein bands at pp130, pp120 and pp116, pp80 and pp70kDa. The phosphorylaton of pp120 was biphasic reaching an initial peak at 2 min, decreasing at 10 min before increasing again between 10 and 60 min. An EC_{50} value (± S.D.) of 52.37 ± 14.nM, n=5, was obtained for LPA-stimulated tyrosine phosphorylation of pp120. Immunoprecipitation experiments revealed that the pp120 band corresponded to focal adhesion kinase pp125^{FAK}. LPA also stimulated protein kinase C activity in Rat-1 fibroblasts which peaked at 2 min (C.P.M./sample Control = 486 ± 16, LPA = 933 ± 56, n=3). Pretreatment with the protein kinase C inhibitor Ro-318220 (Davis *et al.*, 1989) at 10 μM, whilst abolishing LPA stimulated PKC-activity (C.P.M/ sample basal = 539 ± 50, LPA = 1013 ± 66, LPA + Ro-318220 = 469 ± 19, n=3), reduced pp120 tyrosine phosphorylation at 2 and 60 min by only 10-20 % (% max response to LPA, 2 min = 91.7± 10, 60 min 80.7 ± 11, n=6). LPA induced tyrosine phosphorylation of pp80 was reduced by PKC inhibition at 2 min but not at 60 min (% of max response to LPA, 2 min = 38.7 ± 13, 60 min = 122.0 ± 16, n=6).

These results suggest that both PKC dependent and independent pathways are involved in the regulation of protein tyrosine phosphorylation in response to LPA in Rat-1 fibroblasts. The stimulation of focal adhesion kinase pp125^{FAK} suggests this tyrosine kinase may have an important role in regulation of cell growth in response to mitogens.

This work was supported by grants from the Wellcome Trust, Medical Research Council and British Heart Foundation. We would like to thank Dr G. Lawton (Roche products Ltd., Welwyn Garden City, Herts) for the gift of Ro-318220 and Dr T.J. Parsons, University of Virginia for the gift of antibody 2A7.

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32P PROTECTION AGAINST ISCHAEMIC INJURY IN RABBIT MYOCARDIUM 24 HOURS AFTER ADENOSINE A_1 RECEPTOR ACTIVATION

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Recently, we have shown that preconditioning (PC) of myocardium with brief periods of coronary occlusion elicits a resilience to infarction 24 h later. This delayed protection can be inhibited by non-selective adenosine receptor blockade during PC (Baxter & Yellon 1993). We have now examined if a delayed protection could be elicited directly by adenosine A1 receptor activation with the selective agonist 2-chloro-N6-cyclopentyladenosine (CCPA).

Conscious New Zealand White rabbits (2.0-3.0 kg) received intravenous boluses (1.5 ml), through a marginal ear vein, of 0.9% saline (controls), CCPA 25 μg/kg, CCPA 50 μg/kg or CCPA 100 μg/kg. Twenty four hours later, animals were anaesthetised (pentobarbitone sodium supplemented with "Hypnorm") and subjected to a myocardial infarction protocol of 30 min regional ischaemia with 120 min reperfusion. Briefly, the rabbits were ventilated with 100% oxygen and a median thoracotomy was performed. An anterior branch of the coronary artery was ensnared with a silk ligature. After a heparin bolus (1000 i.u.) the artery was occluded for 30 min followed by 120 min of reperfusion. During the procedure heart rate and arterial pressure were monitored continuously via a carotid cannula and rate pressure product (RPP) was calculated. The ischaemic risk zone (R) was determined *ex vivo* with fluorescent microspheres and the infarcted zone (I) using triphenyltetrazolium staining. The volumes of these zones were calculated using computed planimetry. Data are presented as mean ± s.e.m. * $p < 0.05$, ** $p < 0.01$ compared with control group (Dunnett's test).

TABLE 1	control	CCPA 25 μg/kg	CCPA 50 μg/kg	CCPA 100 μg/kg
n	12	6	6	6
Baseline RPPx10^3 (mmHg/min)	19.3 ± 1.3	18.6 ± 2.9	21.8 ± 1.7	18.3 ± 1.3
R (cm^3)	1.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1
I (cm^3)	0.68 ± 0.06	0.40 ± 0.08*	0.43 ± 0.05*	0.27 ± 0.09**
I/R (%)	54.5 ± 2.7	37.1 ± 4.2 **	37.7 ± 2.2**	26.3 ± 5.7**

In separate pentobarbitone-anaesthetised rabbits, CCPA administration caused transient bradycardia and hypotension. Twenty four hours after CCPA pretreatment, systemic haemodynamics were similar in all groups, with no differences in RPP. Although R was similar in all groups, pretreatment with CCPA resulted in marked reductions in I and I/R ratio. In conjunction with our previous report, these data provide further evidence that the second window of protection following brief coronary occlusion, like the early phase of PC protection, may be linked to adenosine receptor activation. We conclude that systemic adenosine A1 receptor activation can trigger a PC-like response evident many hours later, although the molecular basis of the cytoprotection is as yet unknown.

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33P EFFECT OF SITS (4-ACETAMIDO-4-ISOTHIOCYANATO-2,2'-DISULPHONIC ACID) ON SYSTOLIC AND DIASTOLIC FUNCTION BEFORE AND AFTER MYOCARDIAL ISCHAEMIA IN RAT *IN VITRO*

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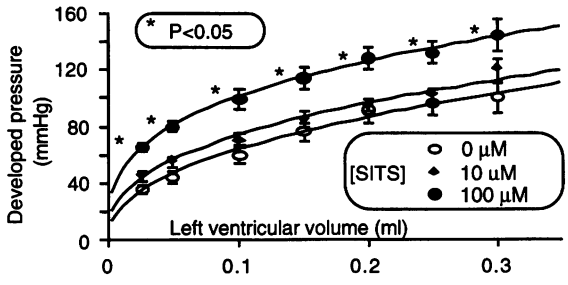
SITS is an anion channel blocker with specific effects on cardiac chloride-bicarbonate exchange (CBE) leading to intracellular alkalinisation (Vaughan Jones 1979). In isolated rat hearts, SITS prevents cardiac arrhythmias elicited by ischaemia and by reperfusion (Curtis 1989) and reduces ventricular excitability (Curtis 1990). These observations raise the possibility that blockade of CBE may represent a new antiarrhythmic mechanism. In the present study we have examined the effects of SITS on cardiac contractile function.

Rat hearts were perfused (Langendorff mode) with solution containing (in mM) NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.0, $MgSO_4$ 1.2, $CaCl_2$ 1.4 and glucose 11.1, pH 7.4, 37°C, and Starling curves were constructed by incremental isochoric volume additions (up to 0.3 ml) to an intraventricular water-filled balloon catheter. Left ventricular developed pressure was calculated as systolic minus diastolic pressure. Heart rate was maintained throughout at 300 beats.min^{-1} by left atrial pacing. Solution was then switched to one containing 0, 10 or 100 µM SITS (n=8/group) in a randomised and blinded fashion. Starling curves were reconstructed 5 min later. In a parallel study, 10 min after switching to test solution, hearts were subjected to 60 min normothermic global ischaemia, then 60 min reperfused with the same solution and Starling curves were repeated. Results were analysed by Dunnett's test (Curtis & Hearse, 1989).

In non-ischaemic myocardium SITS, at 100 but not 10 µM, had a positive inotropic effect at all balloon volumes (Figure). End-diastolic pressure (EDP) was concomitantly increased by 100 µM SITS at volumes ≥0.15 ml ($p<0.05$), although EDP exceeded 10 mmHg only at volumes ≥0.25 mmHg. Following ischaemia and reperfusion, peak developed pressures (mmHg) were low: 28±5, 18±7 ($p>0.05$) and 10±2 ($p<0.05$) with 0, 10 and 100 µM SITS, respectively. In these hearts, at balloon volumes associated with values <10 mmHg prior to ischaemia, diastolic pressures following reperfusion were high: 74±7, 71±5 ($p>0.05$) and 71±1 ($p>0.05$) mmHg, respectively.

In conclusion, in non-ischaemic myocardium SITS enhances systolic contractile function, and slightly diminishes diastolic function whereas, during reperfusion, it exacerbates systolic dysfunction without affecting diastolic dysfunction. Thus, although SITS protects against cardiac arrhythmias during ischaemia and reperfusion, it may jeopardise recovery of function during reperfusion.

(V. Motahar was a recipient of a Wellcome Summer Studentship)



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34P CARDIAC EFFECT OF GLIBENCLAMIDE *IN VITRO* AND *IN VIVO*: A COMPARISON WITH COMPOUNDS RELATED TO AZ-DF 265

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The hypoglycaemic agent (-)AZ-DF 265 inhibits ATP-sensitive potassium (K_{ATP}) channels in pancreatic ß-cells with a potency similar to glibenclamide (GLIB; Ronner *et al.*, 1992). Since GLIB also inhibits K_{ATP} channels in cardiac tissue in vitro, we have evaluated the cardiac effects of (-)AZ-DF 265 and its analogue, (+)-4-[(alpha-Methyl-2-piperidinobenzyl)carbamoylmethyl]=benzoic acid ((+)me AZ-DF 265), in vitro and compared them with GLIB. Since it has been reported that GLIB has antiarrhythmic properties in vivo (Billman *et al.*, 1992), we also compared the effects of these compounds on ischaemic arrhythmias in the anaesthetized cat.

Isolated guinea-pig papillary muscles were stimulated at 1Hz, 5ms pulse width, in Krebs solution at 37°C and changes in isometric tension were measured. Since benzopyrans, such as cromakalim, activate K_{ATP} channels (Sanguinetti *et al.*, 1988), we used the structurally-related compound Ro 31-6930 (Attwood *et al.*, 1991) to induce a negative inotropic response. Ro 31-6930 (2µM), reduced developed tension by 83 ± 3% (from 230.0 ± 26.4mg to 35.6 ± 4.2mg, n=8) after 20 min. Subsequently, GLIB (0.1-100µM for 20 min) concentration-dependently antagonised the effects of Ro 31-6930, with an IC_{50} value and 95% confidence limits of 2.2(1.4-3.4)µM, (n=8). Similarly, (-)AZ-DF 265 and (+)meAZ-DF 265 (0.1-100µM for 20 min) antagonised the negative inotropy induced by Ro 31-6930 with IC_{50} values of 3.8(1.9-7.5)µM, (n=4) and 1.9(1.5-2.3)µM, (n=4), respectively.

Additionally, in anaesthetized cats (Corr *et al.*, 1978) (2.5-5.0kg), the left anterior descending coronary artery was isolated at its origin and occluded for 45 min. Drugs were administered 15 min prior to coronary artery occlusion (CAO) and during the first 15 min of CAO. Ischaemic arrhythmias, arterial blood pressure and heart rate were monitored throughout the experimental protocol. In control animals (n=14), CAO induced ventricular tachycardia (VT, 86%), ventricular fibrillation (VF, 71%) and mortality (MO, 64%). GLIB (3mgkg^{-1}, i.v., n=6) significantly ($P<0.05$) reduced the incidence of VT (17%), VF (0%) and MO (0%). (+)meAZ-DF 265 (3mgkg^{-1}, i.v., n=6) reduced the incidence of VT (33%), VF (17%) and MO (17%) but not significantly. In contrast, (-)AZ-DF 265 (3mgkg^{-1}, i.v., n=6) afforded little protection against lethal ischaemic arrhythmias (100% VT, 50% VF and 33% MO). Neither compound significantly affected arterial blood pressure nor heart rate.

We have shown for the first time that the sulphonylurea, GLIB, reduced the incidence of ischaemic arrhythmias and mortality in this anaesthetized cat model. Although (-)AZ-DF 265, a structurally dissimilar compound with a similar in vitro potency to GLIB, was considerably less effective than GLIB in vivo, (+)meAZ-DF 265 afforded some protection against ischaemic arrhythmias. This difference in antiarrhythmic efficacy between these analogues of AZ-DF 265 may result from differing pharmacokinetic properties of these two drugs.

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35P HUMAN ATRIAL ARRHYTHMIAS PRODUCED THROUGH RECEPTORS POSITIVELY COUPLED TO ADENYLYL CYCLASE

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(-)-Noradrenaline acting through β_1-adrenoceptors (β_1AR), (-)-adrenaline acting through β_2-adrenoceptors (β_2AR) and 5-hydroxytryptamine (5-HT) acting through 5-HT_4 receptors all increase inotropic force, cyclic AMP levels and cyclic AMP-dependent protein kinase (PKA) activity (Kaumann *et al.* 1989, 1990) and evoke rate-dependent arrhythmic contractions (Kaumann & Sanders, 1993*a*,*b*) in isolated human right atrium. We have suggested that atrial arrhythmias are precipitated by myocyte calcium overload resulting from increased inward calcium current caused by PKA-dependent phosphorylation of L-type calcium channels. To test our hypothesis we have compared agonist-stimulated atrial arrhythmias, cyclic AMP levels and PKA activity in the same tissue. Right atrial appendages were obtained from 12 patients undergoing open heart surgery, usually coronary artery bypass surgery, who had been chronically treated with a β blocker. Three to 6 atrial strips obtained from each appendage were paced at 37°C under equilibrium conditions at 1 Hz then at 0.2 Hz for 3 min in the absence or presence of agonist (5 min) before being quick-frozen (liquid N_2) for biochemical assays (Kaumann *et al.*, 1989, 1990). β_1AR were activated by (-)-noradrenaline 10 μM in the presence of ICI 118551 50 nM (to block β_2AR). β_2AR were activated by (-)-adrenaline 10 μM in the presence of CGP 20712A 300 nM (to block β_1AR) (Kaumann & Sanders, 1993*a*). 5-HT_4 receptors were activated by 5-HT 10 μM in the presence of CGP 20712A 300 nM. The effects of prostaglandin E_1 (PGE_1) 20 μM, known to stimulate adenylyl cyclase without causing positive inotropic effects (Kaumann & Birnbaumer, 1974), were also studied. The data are presented in Table 1.

Table 1. Biochemical data (mean ± s.e. mean) and incidence of arrhythmic contractions (n, patient no.) * $P < 0.05$, paired t-test

		Basal (n =11)	Adrenaline (n = 10)	Noradrenaline (n = 6)	5-HT (n = 5)	PGE_1 (n = 8)
Cyclic AMP ($pmol.mg^{-1}$)		25 ± 3	78 ± 5*	98 ± 26*	60 ± 10*	45 ± 5*
PKA activity ratio		0.27 ± 0.02	0.53 ± 0.02*	0.53 ± 0.06*	0.44 ± 0.03*	0.35 ± 0.01*
Arrhythmic	1 Hz	0/11	7/10	3/6	2/5	0/8
contractions	0.2 Hz	1/11	9/10	5/6	2/5	0/8

The incidence of arrhythmic contractions appears to be a function of the degree of PKA stimulation. PGE_1 may stimulate PKA in a pool that is not relevant to arrhythmias. We thank the surgeons of Papworth Hospital for the supply of atrial tissues.

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36P THE DIFFERENTIAL EFFECT OF CAPTOPRIL ON THE CARDIAC HYPERTROPHIC AND INOTROPHIC EFFECTS OF CHRONIC NORADRENALINE TREATMENT IN RATS

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Overactivity of the sympathetic nervous system is one of the components of heart failure and the beneficial effect of captopril in its treatment has been linked to the prevention of myocardial β_1-adrenoceptor downregulation (van Wijngaarden *et al*, 1992). Chronic administration of noradrenaline in experimental animals has been reported to cause subsensitivity to acutely administered noradrenaline (Brown *et al*, 1992). The aim of this study was to determine whether pre-treatment with captopril modified the myocardial and haemodynamic consequences of chronic administration of noradrenaline in rats.

Male Sprague-Dawley rats were anaesthetised with halothane and 1 or 2 osmotic mini-pumps were inserted into a subcutaneous pocket. Rats were administered either (±) noradrenaline (0.15 mg $kg^{-1}hr^{-1}$; n=9), captopril (0.25 mg $kg^{-1}hr^{-1}$; n=6), noradrenaline & captopril (n=5) or vehicle (acid saline; n=8). 28 days post-implantation animals were anaesthetised with sodium pentobarbitone and prepared for the measurement of heart rate, arterial blood pressure and left ventricular pressure (via a Millar Microtip transducer) from which the indices of contractility dP dt^{-1}_{max} and dP $dt^{-1}P^{-1}$ were calculated. Dose-response curves were constructed for acutely administered noradrenaline (0.1-5 μg kg^{-1}) and for calcium chloride (0.25-40 mg $kg^{-1}hr^{-1}$).

Chronic noradrenaline administration caused left, but not right, ventricular hypertrophy (left ventricle & septum dry weight to body weight ratio was 0.567 ± 0.015 and 0.462 ± 0.012 in treated and control animals respectively; $P<0.05$) an effect which was attenuated by co-administration of captopril (left ventricle & septum dry weight to body weight ratio was 0.511 ± 0.013; ns). Animals administered noradrenaline also had significantly elevated systolic arterial pressures (194 ± 11 vs 130 ± 6 mmHg), systolic left ventricular pressures, heart rates (458 ± 13 vs 389 ± 15 beats min^{-1}) and contractile indices (dP dt^{-1} P^{-1} was 202 ± 29 vs 91 ± 3 s^{-1}) compared to controls ($P<0.05$). Captopril alone reduced diastolic arterial pressure (53 ± 3 vs 86 ± 6 mmHg) and systolic left ventricular pressure (78 ± 3 vs 123 ± 4 mmHg). Concomitant administration of captopril with noradrenaline prevented the increases in systolic arterial pressures (123 ± 34 mmHg) and systolic left ventricular pressures. In contrast, captopril did not prevent the increases in heart rate (496 ± 17 beats min^{-1}) or the contractile indices (dP $dt^{-1}P^{-1}$ was 261 ± 41 s^{-1}) induced by chronic treatment with noradrenaline alone. Chronic administration of noradrenaline resulted in a reduced responsiveness to acutely administered noradrenaline an effect which was not altered by captopril. The positive inotropic effects of calcium chloride were similar in all treatment groups.

These results demonstrate that chronic captopril treatment attenuates noradrenaline-induced cardiac hypertrophy and this is associated with a vasodepressor effect. Captopril did not modify the noradrenaline-induced subsensitivity to acutely administered noradrenaline.

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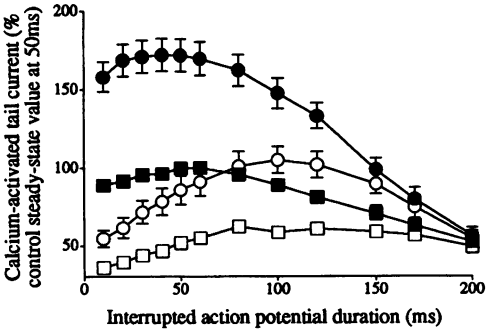
S. K. Laycock held an SERC studentship; running costs for the project were provided by Bristol-Myers Squibb.

37P INFLUENCE OF ISOPRENALINE ON THE SARCOPLASMIC RETICULUM CONTRIBUTION TO CALCIUM TRANSIENTS IN MYOCYTES ISOLATED FROM GUINEA-PIG VENTRICLE

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Isoprenaline is known to increase calcium transients in cardiac muscle cells (e.g. Endoh & Blinks, 1988). The purpose of the present experiments was to investigate how increased uptake of calcium into sarcoplasmic reticulum stores may contribute to this effect.

Myocytes were isolated from guinea-pig ventricle and bathed in a balanced salt solution containing 2.5 mM calcium. Action potentials (stimulation frequency 1-3.3 Hz) were interrupted by application of a voltage clamp to -70 mV. Calcium transients were constructed from the magnitude of inward currents thought to be carried predominantly by Na:Ca exchange (see Terrar & White, 1989). Repeated application of the voltage clamp to a potential more negative than the action potential plateau caused depletion of intracellular stores, by enhancing calcium extrusion through Na:Ca exchange across the sarcolemma at the expense of stores uptake (Terrar & White 1989).



The filled squares in the figure show the calcium transient when the stores were in a steady state (1 Hz). Open squares show the calcium transient when the stores were depleted following repeated interruption of action potentials by the voltage clamp. Corresponding observations in the presence of 5 nM isoprenaline are shown as filled (steady state) and open (depleted) circles. It is clear that isoprenaline greatly enhanced the peak of the calcium transient (by 72±10%, n=16 cells, $p<0.05$). The contribution of the stores to this transient (shown as the difference between the depleted and steady state components which is most marked early in the transients) was also increased by isoprenaline ($p<0.05$ for the measurements at 10 ms, n=16). The effects of isoprenaline on calcium transients were greatly reduced when stores were first depleted by exposure to 2 µM ryanodine.

The observations are consistent with enhanced uptake of calcium into the sarcoplasmic reticulum providing a major contribution to the increased calcium transient in the presence of isoprenaline.

This work was supported by the Wellcome Trust.

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38P AGE-DEPENDENT DECREASE OF β-ADRENOCEPTOR FUNCTION IN HUMAN RIGHT ATRIUM

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With increasing age cardiac β-adrenoceptor (AR) function declines (Van Brummelen et al., 1981; Scarpace et al., 1991). To study possible mechanisms underlying this process in this study we assessed in right atrial appendages of 52 patients without apparent heart failure β-AR number, adenylyl cyclase (AC) activity and α-subunits of G_s- and G_i-protein.

Right atrial appendages were obtained from 20 children undergoing open heart surgery and from 32 patients undergoing coronary artery bypass grafting; none of the patients had been treated with β-AR antagonists for at least 6 weeks before operation. Patients were divided into 3 age-groups: A (0-19 yrs, n=20), B (20-50 yrs, n=9), C (51-83 yrs, n=23). β-AR number was assessed by (-)-[^{125}I]iodocyanopindolol (ICYP) binding, AC-activity as conversion of [^{32}P]ATP to [^{32}P]cAMP during a 10 min incubation at 30°C (Schranz et al., 1993). The α-subunits of G_s and G_i were quantified by quantitative Western blotting with the specific RM/1 and AS/7 antisera, respectively, followed by [^{125}I]-protein A detection.

β-AR number was in the 3 groups not significantly different, but showed a tendency to decline from A to C (A: 68±9 fmol ICYP/mg protein, B: 62±8 fmol/mg, C: 57±10 fmol/mg). In contrast, basal, 10 µM GTP-, 100 µM isoprenaline-, 10 mM NaF-, 100 µM forskolin- and 10 mM Mn^{2+}-stimulated AC activity were in A significantly higher than in B and declined further in C. Similarly, 100µM terbutaline-, 100µM histamine- and 100µM serotonin-stimulated AC activity significantly declined from A to C. We observed no significant differences in the 3 groups for immunodetectable $G_{s\alpha}$, but $G_{i\alpha}$ was in C significantly higher than in A (37kDa band; A: 2521±136 cpm/mg protein, C: 3068±259 cpm/mg, $0.1>P>0.05$; 43kDa band: A: 3720±199 cpm/mg, C: 4677±407 cpm/mg, $P<0.05$). Moreover, there was a weak, but significant ($P<0.05$) positive correlation between $G_{i\alpha}$ and age of patients (37kDa band: $r=0.282$, 43kDa band: $r=0.303$).

We conclude that the decrease in cardiac β-AR function with increasing age is due to impairement of the catalytic unit of AC; an increase in amount (and function) of $G_{i\alpha}$ might further contribute to this reduced β-AR function.

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39P EXAMINATION OF TRANSREGULATION OF β-ADRENOCEPTORS AND MUSCARINIC CHOLINOCEPTORS IN RAT HEART

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Chronic stimulation of β-adrenoceptors (β-AR) leads to desensitisation and receptor down-regulation (Sibley *et al.*, 1985). Recent studies suggest that muscarinic cholinoceptors (mAchR) which are negatively linked to adenylate cyclase may also be transregulated (Marquetant *et al.*, 1992). This study examines the effect of chronic (-)-isoprenaline (iso) infusion on β-ARs and mAchRs in regions of the rat AV conducting system and myocardium using autoradiography, and in ventricle using homogenate receptor binding techniques.

Rats were chronically infused with iso (450 μg/kg/hr) or vehicle (1 mM HCl) for 14 days. Daily systolic blood pressure (SBP) and heart rate (HR) recordings showed that SBP in iso treated rats was reduced for 10 days before returning to control levels, while HR was significantly increased over the 14 day period($P<0.05$, ANOVA). β-ARs were labelled with (-)-[^{125}I]-cyanopindolol (CYP, 50 pM) in the presence or absence of CGP 20712A (100 nM, β_1-selective) or (-)-propranolol (1 μM, non-selective). mAchRs were labelled with [^{3}H]-N-methylscopolamine (NMS, 1 nM) and non-specific binding determined with atropine (1 μM). Quantitative autoradiography showed that iso treatment reduced β_1-ARs by 43.3-79.0% and β_2-ARs by 86.0-98.5% ($P<0.05$, n=6) in atrial and ventricular myocardium, pacemaker and conducting regions with the greatest reductions observed in the AV conducting regions. Homogenate binding studies using ventricle from the same animals revealed a reduction in total membrane β-ARs from 23.1±1.6 fmol/mg protein in control to 13.0±3.0 fmol/mg protein in iso treated rats ($P<0.05$, n=6). Receptor proportions (β_1:β_2) in control rats were 71.2:28.8% and in iso treated rats 86.1:12.9%, with K_D values for (-)-[^{125}I]-CYP of 11.8±1.6 pM and 23.0±2.2 pM respectively. Quantitative autoradiography of mAchRs with [^{3}H]-NMS showed no change in density in any region ($P>0.276$, n=6). Homogenate binding showed a higher density of mAchRs in membranes from iso treated rats (control 74.8±6.2 fmol/mg protein, iso 119.3±11.0 fmol/mg protein, $P<0.05$, n=6), with no change in the K_D (control 534.0±45.7 pM; iso 539.0±35.6 pM), suggesting movement of pre-existing receptors to a compartment accessible by the ligand.

In conclusion, iso infusion reduced total cellular and membrane bound β_1- and β_2-adrenoceptors, and initiated translocation of mAchRs with no evidence for new receptor synthesis.

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40P CHARACTERISTICS OF β-ADRENOCEPTORS IN CARDIAC TISSUE FROM SPONTANEOUSLY HYPERTENSIVE RATS WITH STREPTOZOTOCIN- INDUCED DIABETES MELLITUS

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Diabetes mellitus and hypertension are problems which often coincide. In the present study we investigated the characteristics of cardiac ß-adrenoceptor binding sites in an animal model of combined diabetes mellitus and hypertension.
Spontaneously hypertensive (SHR) and normotensive Wistar-Kyoyo rats (WKY) at an age of 14 weeks were made diabetic by a single injection of streptozotocin (60 mg/kg) in a lateral tail vein. After 8 weeks the rats were anaesthetized and the mean arterial blood pressure and the plasma glucose concentration were determined. Subsequently the hearts were removed and binding assays were performed in membrane preparations of cardiac left ventricular tissue by means of (-)-[^{125}I]-iodocyanopindolol. The density (B_{max}) and the dissociation constants (K_D) were evaluated by Scatchard-analysis. In order to determine the ratio between $ß_1$- and $ß_2$-adrenoceptor subtypes competition experiments with the unlabeled $ß_2$-selective ligand ICI 118,551 were performed. The ratio $ß_1/ß_2$ was calculated by subjecting the concentration-inhibition data to a non-linear curve fitting procedure with the assumption of two independent binding sites.
Mean arterial blood pressure (MAP), blood glucose concentrations, B_{max}, K_D and the $ß_1/ß_2$-ratio are given in table 1 as mean values ± S.E.M. (n). Significance was calculated by ANOVA analysis of variances. $P < 0.05$ was considered to be significant.

Table 1	WKY control	WKY diabetic	SHR control	SHR diabetic
MAP (mmHg)	104 ± 2.5 (28)	112 ± 3.7 (31)	173* ± 3.8 (36)	164* ± 3.9 (33)
glucose (mmol/l)	6.93 ± 0.25 (44)	25.65‡ ± 0.39 (54)	7.64 ± 0.22 (48)	24.36‡ ± 0.49 (49)
B_{max} (fmol/mg prot.)	52 ± 3.2 (6)	36‡ ± 3.9 (6)	53 ± 4.9 (6)	35‡ ± 5.0 (7)
K_D (nmol/l)	0.11 ± 0.05 (6)	0.04 ± 0.01 (6)	0.04 ± 0.01 (6)	0.12 ± 0.04 (7)
$ß_1/ß_2$ (%)	74.5 / 25.5 ± 2.7 (4)	71.2 / 28.8 ± 6.2 (8)	67.0 / 33.0 ± 2.0 (5)	63.3 / 36.7 ± 2.0 (8)

* denotes a significant difference compared to the particular normotensive group
‡ denotes a significant difference compared to the particular normoglycaemic group

The model of streptozotocin-induced diabetes in spontaneously hypertensive rats appears to be suitable to investigate these pathological entities separately as well as combined.
The total density of cardiac ß-adrenoceptors is significantly decreased by diabetes but not by hypertension. This reduction of the receptor density is not due to a decrease of a particular ß-adrenoceptor subtype.

41P HISTAMINE RECEPTORS IN THE HEART: MOLECULAR CHARACTERISATION, PHYSIOLOGY AND PHARMACOLOGY

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Histamine effects in the heart are mediated through the stimulation of histamine H1- and H2-receptors. The simultaneous activation of cardiac H1- and H2-receptors result in: increased heart rate, force of contraction, automaticity, coronary blood flow, and decreased atrioventricular nodal conduction velocity. Histamine, through H3-receptors, might be also involved in attenuation of noradrenaline release in the heart (Wolff & Levi, 1986). H1- and H2-receptors, which have already been cloned, are two independent molecular entities differing in the pathways of transmembrane and intracellular signalling. H1-receptors are connected to the activation of phosphoinositidase C, H2-receptors stimulate adenylyl cyclase. Our experiments carried out in this study indicated also the difference in the characteristics of their binding sites. Using radioligand binding assay with [^{3}H]-mepyramine and [^{3}H]-tiotidine as radiolabels the molecular properties of H1- and H2- receptors were identified and characterized in guinea pig myocardium. The mapping with [^{3}H]-mepyramine revealed the existence of high and low affinity binding sites for the ligand with an equilibrium dissociation constant (K_D) of 0.4 ± 0.1 nM and a maximal density of binding sites (B_{max}) of 100 ± 9 fmol/mg of protein for high and a K_D of 4.5 ± 0.6 nM and a B_{max} of 466 ± 66 fmol/mg of protein for low affinity binding sites (Kržan et al, 1993). [^{3}H]-mepyramine binding was displaced by competitors in rank order typical for the functional H1-receptor: mepyramine > triprolidine > histamine > 2-methylhistamine > 4-methylhistamine. With [^{3}H]-tiotidine we successfully mapped H2-receptors in guinea pig myocardial membranes. In this tissue a single population of binding sites with a K_D of 1.0 ± 0.07 nM and a B_{max} of 27 ± 2 fmol/mg of protein was found (Kržan et al, 1993). The rank order of competitor potencies was: famotidine > cimetidine > triprolidine > mepyramine > histamine > 4-methylhistamine > impromidine - typical for the functional H2-receptor.

The molecular properties of H1- and H2-receptors elucidated in our study revealed that the density of H1-receptors is significantly higher than the density of H2-receptors in guinea pig ventricular myocardial membranes. The ratio is 4:1. Furthermore, for H1-receptor high and low affinity binding sites exist, whereas for H2-receptors only high affinity sites are present in the heart. The results of inhibition binding experiments carried out with different H1 and H2-specific agonists and antagonists support our conclusion that the specific [^{3}H]-mepyramine binding in this study represents binding to H1-receptors and that H2-receptors were specifically labelled with [^{3}H]-tiotidine. In a comparative study (Čarman-Kržan & Kržan, 1993) the binding properties of cardiac H1-receptors were shown to be different from vascular H1-receptors. An unique cardiac isoform of the H1-receptor was found using autoradiographic studies (Ruat et al. 1990) and no H1-receptor mRNA was found in bovine atrium, both supporting the observation, that cardiac H1-receptors are different from other peripheral H1-receptors.

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* this is an abstract of the winning essay for G.B. West Memorial Prize 1993

42P UPTAKE OF RENIN, ANGIOTENSINOGEN AND ANGIOTENSIN I AND II BY THE ISOLATED RAT HEART

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The renin-angiotensin system (RAS) has traditionally been viewed as an endocrine system. Circulating kidney-derived renin (R) acts on circulating liver-derived angiotensinogen (Ao), thereby forming angiotensin I (AI). AI is converted to angiotensin II (AII), a potent vasoconstrictor, by endothelial-bound angiotensin converting enzyme (ACE). Recently however, it has been shown that AI and AII are also generated locally in various tissues, for instance in the heart and blood vessels. The RAS components necessary for local generation are either synthesized in these tissues (Dzau *et al.*, 1986) or taken up from the circulation (Admiraal *et al.*, 1990; Danser *et al.*, 1992). The aim of the present study was to investigate the uptake of RAS components by the rat heart, using a modified Langendorff technique allowing the separate collection of coronary (CE) and interstitial (ISF) effluents (De Deckere & Ten Hoor, 1977). Infusions of R (n=7), Ao (n=5), AI (n=7) and AII (n=5) were given to isolated rat hearts in the presence of the AT_1 receptor antagonist losartan (final concentration 10^{-6} M), and the various RAS components were measured in CE and ISF before, during and after these infusions.

Prior to their infusion, R, Ao, AI and AII were below the detection limit in both CE and ISF. R and Ao entered the ISF slowly during infusion (steady-state levels in 10 and > 15 min, respectively) and they could still be detected in ISF but not in CE 10 min after the infusion had been stopped. Steady-state R levels in ISF were 2-3 times higher than in CE. Steady state Ao levels in ISF were equal to those in CE. Steady-state levels for both AI and AII were reached within several min after starting their infusion, and AI and AII disappeared from ISF within 2-3 min after the infusion had been stopped. Steady-state AI and AII levels in ISF were 25 % and 55 % of CE levels, respectively. AI infusion resulted in the appearance of AII in CE and ISF; the steady-state AII levels during AI infusion were comparable in the two compartments (Table 1).

Table 1. Steady state levels of RAS components in CE and ISF during their infusion (mean and SD; * $p < 0.05$, ISF vs. CE).

	R (fmol AI/min·ml)	Ao (pmol/ml)	AI (pmol/ml)	AII during AI (pmol/ml)	AII (pmol/ml)
CE	256 ± 267	12.4 ± 2.6	2.9 ± 1.7	0.4 ± 0.3	3.3 ± 1.7
ISF	472 ± 365*	9.4 ± 4.7	0.8 ± 0.8*	0.5 ± 0.2	1.7 ± 0.8*

Conclusion: The heart is capable of extracting R from the circulation, possibly by an active process, whereas Ao seems to enter the cardiac interstitium by passive diffusion. Plasma AI and AII rapidly enter the interstitium. Part of AII in the cardiac interstitium is derived from circulating AI, which is converted to AII at sites other than the coronary endothelium.

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43P CAPTOPRIL ENHANCES VENTRICULAR RELAXATION VIA ENDOGENOUS BRADYKININS

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Recent studies have shown that nitric oxide (NO) can modulate myocardial contraction in isolated cardiac myocytes, papillary muscles and the intact ventricle (Shah & Lewis, 1993). NO increases myocardial cyclic GMP (cGMP), which appears to reduce myofilament response to Ca^{2+}, thereby hastening the onset of relaxation, with little change in early contractile parameters. Bradykinin (BK) is a potent agonist for the release of NO from endothelial cells. Endothelial cells can themselves release BK, which could act in an autocrine and paracrine manner to induce NO release. Captopril (Cap) inhibits angiotensin-converting enzyme, which is also responsible for the degradation and inactivation of BK. We examined the effects of BK and Cap on ventricular function in isolated ejecting ('working') guinea-pig hearts (constant preload [10 cmH_2O], afterload [70 cmH_2O] and heart rate; recirculating Krebs buffer; 37°C). We investigated the role of endogenous BK in the response to Cap using HOE 140, which is a potent and selective antagonist of BK B2 receptors. Left ventricular pressure (LVP) fall, measured by a high-fidelity 2F micromanometer-tipped catheter was biphasic and well characterised by two monoexponential time constants; (T_E) for early relaxation and (T_L) for late relaxation.

	Control (absolute baseline values)	BK 1nM	BK 100nM	Cap 1μM	Cap+HOE 140 (10nM)
CF	1.9±1.4 (11.3±0.5 $mlmin^{-1}$)	37.3±4.7*	63.0±3.1*	1.6±2.8	-3.8±1.7
T_E	-1.5±2.0 (218.7±10.4 ms)	-8.7±1.5*	-19.9±2.5*	-15.0±1.2*	-6.3±1.3†
T_L	6.1±3.0 (20.3±1.2 ms)	-4.2±2.3	13.0±2.5*	7.8±2.5	0.9±5.5
dP/dt	-2.6±1.1 (2.0±0.1 $mmHgms^{-1}$)	1.3±1.2	-6.3±1.3*	-2.3±1.1	-3.6±1.0

Percent changes ± s.e.mean in peak coronary flow (CF), T_E, T_L and peak dP/dt. n≥6; *$p<0.05$ c.f. control; †$p<0.05$ Cap + HOE 140 c.f. Cap alone. The baseline values for other groups were not significantly different to the control group.

Thus, BK increased the rate of early LV relaxation (reduced T_E) with little change in 'systolic' parameters e.g. peak dP/dt and LVP or end-diastolic LVP (data not shown). Cap induced similar changes in LV relaxation and these were significantly inhibited by HOE 140, indicating that the response to Cap was mediated,.at least in part via the action of endogenous BK on B2 receptors. Interestingly Cap did not alter CF, which may reflect the site of release of BK. This mechanism may contribute to the beneficial effects of Cap in the treatment of heart failure.

Shah, A.M. & Lewis M.J. Trends Cardiovasc Med 1993;3:98-103.

44P COMPARISON OF THE CARDIOVASCULAR EFFECTS OF THE ANGIOTENSIN AT_1 RECEPTOR ANTAGONISTS, GR138950 AND LOSARTAN, WITH ENALAPRIL IN CONSCIOUS RENAL HYPERTENSIVE RATS

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The cardiovascular profile of the potent and selective angiotensin AT_1 receptor antagonist GR138950 (Hilditch *et al.*, 1993) has been investigated in freely moving conscious normotensive (NT) rats and renal artery ligated hypertensive (RALH) rats. In addition, the cardiovascular effects of losartan and enalapril have been compared to those of GR138950 in conscious RALH rats.

Hypertension was induced in male AH/A rats (300-400g) by ligation of the left renal artery and animals were used 6 days after ligation (Travers & Hilditch, 1992). Catheters were implanted into a jugular vein for drug administration and into a tail artery for the measurement of blood pressure, from which heart rate was derived. Administration of vehicle caused little change in mean blood pressure (MBP) and heart rate (HR) in NT and RALH rats. GR138950 (1 mg kg^{-1}) did not significantly ($P>0.05$) affect MBP and HR in NT rats. In contrast, GR138950 (0.1 and 1 mg kg^{-1}) caused a dose-dependent reduction in MBP associated with a tachycardia in RALH rats (Table 1). Losartan (3 mg kg^{-1}) and enalapril (1 mg kg^{-1}) produced similar falls in MBP with concomitant tachycardia. Peak tachycardia was observed within 10 min of administration, whereas maximum pressure changes occurred between 5-7h. The tachycardia caused by GR138950 (1 mg kg^{-1}) was significantly ($P<0.01$) attenuated in animals pretreated (20 min) with atenolol (0.5 mg kg^{-1} + 0.2 mg kg^{-1} h^{-1}; i.v.) and was abolished by a combination of atenolol and atropine methylnitrate (0.5 mg kg^{-1} + 0.1 mg kg^{-1} h^{-1}; i.v.).

Table 1: Maximum changes (mean ± s.e.mean) 0-10 min and 5-7 h following intravenous administration of test compound (n=5-7).

		NT rat	Renal Artery Ligated Hypertensive rat					
		GR138950	GR138950	GR138950	GR138950 1 mg kg^{-1}		Losartan	Enalapril
		1 mg kg^{-1}	0.1 mg kg^{-1}	1 mg kg^{-1}	Atenolol	Atenolol+Atropine	3 mg kg^{-1}	1 mg kg^{-1}
Δ MBP (mmHg)	0-10 min	-4 ± 1	-27 ± 5**	-54 ± 7**	-41 ± 2	-47 ± 4	-44 ± 6**	-48 ± 4**
	5-7 h	-9 ± 4	-60 ± 7**	-95 ± 9**	-83 ± 6	-95 ± 6	-94 ± 7**	-80 ± 9**
Δ HR (beats min^{-1})	0-10 min	18 ± 6	95 ± 25**	109 ± 8**	37 ± 8†	3 ± 6†	183 ± 27**	159 ± 28**
	5-7 h	19 ± 9	16 ± 19	48 ± 15*	23 ± 9	-48 ± 10†	102 ± 22**	86 ± 33**

Baseline values; NT rat MBP 101 ± 3 mmHg, HR 346 ± 16 beats min^{-1}; RALH rat MBP 164-177 mmHg, HR 347-382 beats min^{-1}.
* $P<0.05$, ** $P<0.01$ compared (Student's unpaired *t* test) with vehicle controls. † $P<0.01$ compared with GR138950 (1 mg kg^{-1}; RALH rats).

These results show that angiotensin AT_1 receptor antagonists or an ACE inhibitor reduce blood pressure and cause tachycardia in conscious RALH rats. The tachycardia appears to be mediated by sympathetic stimulation and inhibition of vagal tone and is likely to be reflex in origin. Previous studies have not demonstrated a tachycardia with compounds of this type in RALH rats (Wong *et al.*, 1990; Hilditch *et al.*, 1993).

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45P EARLY AND LATE EFFECTS OF ANGIOTENSIN II SUBTYPE 1 RECEPTOR BLOCKADE ON HAEMODYNAMICS AND CARDIOVASCULAR REMODELLING IN SPONTANEOUSLY HYPERTENSIVE RATS

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In spontaneously hypertensive rats (SHRs), cardiac and vascular remodeling - whether a consequence or the cause of hypertension - progressively develops together with the rise in blood pressure. Limitation of this remodeling by *non selective* blockade of circulating and tissue renin-angiotensin systems (RAS) with angiotensin I-converting enzyme inhibitors strongly suggests that angiotensin II (Ang II) is crucially involved in the process. The aim of this study was to *selectively* readdress this issue by investigating the long term hemodynamic and morphological effects of a new Ang II subtype 1 (AT1) receptor antagonist, SR 47436 /BMS-186295(SR/BMS) (Cazaubon et al., 1993), in young SHRs both during and after the treatment period.

Two groups of SHRs were given SR/BMS (60 mg/kg/day, n=115) or distilled water (controls, C, n=115) by gavage from their 4th to 20th weeks of age. Treatment was then stopped but observations continued for another 8 week period. At selected times (8, 14, 20 and 28 weeks of age), the systemic and regional hemodynamic (radioactive microsphere technique) as well as the cardiac and vascular morphological parameters (automatic image analysis) were investigated and compared in the two groups of animals.

SR/BMS opposed genetic hypertension development (GHD) during the treatment period (systolic blood pressure : 182 vs 222 mmHg in C, $p<0.001$ at 20 weeks). This was accompanied by an early (starting at 8 weeks) and strong limitation of the age-related rise in total peripheral (-23% vs C, $p<0.01$ at 20 weeks) and regional vascular resistances (-26 to -40% vs C, depending upon the vascular bed, $p<0.05$, at 20 weeks). From the morphological view point, SR/BMS strongly opposed the development with age of aortic wall hypertrophy as evidenced by significant reductions in media thickness (-16% vs C, $p<0.001$ at 20 weeks) and in wall to lumen ratio and by a significant increase in aortic nuclear density. SR/BMS also limited the development of vascular fibrosis (collagen : -17% vs C at 20 weeks, $p<0.05$). At the cardiac level, SR/BMS opposed myocardial hypertrophy (heart weight/body weight ratio : -14% vs C, $p<0.01$ at 20 weeks) and subendocardial fibrosis (-48% vs C, $p<0.001$ at 20 weeks). All these vascular and cardiac morphological effects of SR/BMS were delayed in their onset (14 or 20 weeks) as compared to the hemodynamic ones. Eight weeks after treatment withdrawal and although systemic and regional hemodynamic parameters had returned to control values, blood pressure remained significantly decreased in the SR/BMS group (207 vs 228 mmHg in C, $p<0.001$). Morphologically, aortic wall hypertrophy and fibrosis as well as cardiac fibrosis remained significantly decreased but myocardial hypertrophy was no longer significantly reduced.
We conclude that in SHRs (a) the selective interruption of the RAS through AT1 receptor blockade prevents GHD, (b) the early limitation of the age-related increase in total and regional vascular resistances initiates this prevention, (c) the delayed interruption of the vascular and cardiac remodeling later on also contributes, and (d) the sustained antihypertensive effects observed after treatment withdrawal are related to the maintained suppression of the vascular rather than of the cardiac remodeling.

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46P ALTERATIONS IN FUNCTION AND MORPHOLOGY OF THE RABBIT SUBCLAVIAN ARTERY FOLLOWING ANGIOPLASTY

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Restenosis is a major complication of balloon angioplasty (Ledley & Williams, 1990). However, it is not known whether the morphological changes lead to alteration in function. Our aim was to correlate morphology with contractile and relaxant responsiveness in a model of restenosis initiated by angioplasty. Male New Zealand White rabbits (12 wks old) were fed a high cholesterol diet (1%) for 4 weeks prior to angioplasty. Angioplasty of the left subclavian artery involved two 30s inflations of a 3.0mm balloon catheter to 14atm followed by a single inflation to 12atm, during which the balloon was withdrawn by half its length. Animals were returned to a normal diet and sacrificed at 2, 7, 15 or 30 days after angioplasty. The left and right subclavian arteries were removed and each was cut into six 3-4 mm rings. Half of these were used for morphological analysis (using computerised planimetry), the rest for isometric force measurements. U.S. trichrome staining indicated the development of a neointima, in the angioplastied artery only, between 2 and 7 days after the operation, which reached a peak (12.1 $\pm$ 3.2% of the area within the external elastic lamina. (n=6)) by 15 days and then ceased to increase in size. Immunohistological staining determined that the neointima comprised mainly smooth muscle cells, with some macrophage/foam cells and extracellular matrix. Additionally endothelial cover was found to decrease to its lowest level one week following angioplasty, with subsequent endothelial hyperplasia. Concentration response curves were produced to KCl (15-100mM) and, after contraction with 1mM 5-HT, to Carbachol (0.01-5.0mM), A23187 (0.01-1.0mM) and Sin-1 (0.01-1.0mM). The maximum relaxation induced by carbachol (Table 1) shows a severe hypercholesterolaemia-induced endothelial dysfunction. This was gradually reversed in the control vessels on return to a normal diet (data not shown) but did not start to reverse in angioplastied vessels until the 15 day timepoint. This was also seen with A23187 but not with Sin-1 (Table 1). The production of the neointima produced an increase in sensitivity to KCl which was most marked at 30 days but was not statistically significant.

Table 1. Max. relaxation of the angioplastied artery as a percentage of 5-HT induced contraction.

Agonist.	Normochol.	Hyperchol.	2 Day.	7 Day.	15 Day.	30 Day.
Carbachol	68.4 $\pm$ 8.9	16.4 $\pm$ 8.8*	29.7 $\pm$ 6.8	16.3 $\pm$ 8.8*	27.5 $\pm$ 12.6*	41.3 $\pm$ 9.4
A23187	56.9 $\pm$ 7.3	16.4 $\pm$10.7*	49.0 $\pm$ 9.0	38.4 $\pm$ 16.2	39.6 $\pm$ 7.8	39.1 $\pm$ 7.7
Sin-1	57.6 $\pm$ 5.8	70.4 $\pm$ 6.4	53.4 $\pm$ 3.4	58.1 $\pm$ 8.8	58.6 $\pm$ 6.8	57.2 $\pm$ 11.5

Values are given as means $\pm$ sem, n=3-6 for all values .*$P < 0.05$ compared to normocholesterolaemic control using analysis of variance oneway with a Tukey multiple range test.

Restenosis following angioplasty was caused by the development of a neointima containing smooth muscle cells, which may account for the increase in sensitivity of the angioplastied vessel to KCl after 30 days. The high cholesterol diet impairs EDRF mediated vasodilation, apparently acting beyond the muscarinic receptors. This impairment is not exacerbated by angioplasty but recovery of function is delayed. Hypercholesterolaemia and angioplasty do not appear to inhibit the ability of the artery to relax to a nitric oxide donor.

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P.Hadoke holds a Strathclyde University studentship. The work was sponsored by Ciba Geigy Pharmaceuticals (Horsham).

47P FOUR HUMAN SOMATOSTATIN (SRIF) RECEPTORS (SSTR-1 TO SSTR-4) ARE NEGATIVELY COUPLED TO ADENYLYL CYCLASE

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At least five cDNAs coding for SRIF receptors have been cloned of which four are present in the brain (Kaupmann et al, 1993): SSTR-1 (Yamada et al, 1992), SSTR-2 (Kluxen et al, 1992), SSTR-3 (Yasuda et al, 1992) and SSTR-4 (Bruno et al, 1992). Human SRIF receptors were expressed in Human Embryonic Kidney (HEK 293) and Chinese Hamster Ovary (CHO) cells and their ability to modulate adenylyl cyclase activity investigated. Stimulation of SSTR-2, SSTR-3 and SSTR-4 receptors produced inhibition of 10 μM forskolin-stimulated cAMP production (FSAC) in intact HEK 293 (not shown, Kaupmann et al, 1993) and CHO cells (see table 1). Stimulation of SSTR-1 receptors did not affect basal or FSAC in CHO cells, but inhibited FSAC in HEK 293 cells (table 1).

Table 1: Inhibition of forskolin-stimulated cAMP production via SRIF receptors (pEC_{50}, -log mol/l; Emax, % inhibition; n ≥ 3).

	SSTR-1		SSTR-2		SSTR-3		SSTR-4	
Drug	pEC_{50}	Emax	pEC_{50}	Emax	pEC_{50}	Emax	pEC_{50}	Emax
SRIF-14	9.19	68	8.81	51	10.20	83	10.00	58
SRIF-28	9.01	67	9.07	54	9.58	88	9.89	57
octreotide	<6.00	21*	9.38	61	8.09	89	7.07	39
seglitide	<6.00	21*	9.30	50	7.23	84	<6.00	15*

* denotes inhibition at 1 μM. The rank order of potency of the agonists tested in second messenger experiments were similar to their respective rank order of affinity determined in radioligand binding studies using recombinant receptors expressed in either cell system. The data suggest that all four SRIF receptors expressed in the brain may modulate adenylyl cyclase activity in contrast to initial claims (Bell & Reisine, 1993). Two splice variants of SSTR-2 have been described (Vanetti et al, 1992): our own data were generated with the unspliced form, suggesting that splicing is not required for SSTR-2 to couple to adenylyl cyclase.

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48P *IN VITRO* AND *IN VIVO* PHARMACOLOGY OF THE NON-PEPTIDE ANTAGONIST AT TACHYKININ NK_2-RECEPTORS, GR159897

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We report the *in vitro* and *in vivo* pharmacology of GR159897 ((R)-1-[2-(5-fluoro-1H-indol-3-yl)ethyl]-4-methoxy-4-[(phenylsulfinyl)methyl]-piperidine), a novel, low molecular weight, non-peptide antagonist at tachykinin NK_2-receptors. We determined the binding affinity of GR159897 at NK_2-receptors from human ileum, recombinantly expressed in CHO cells (hNK_2-CHO; Hagan et al., 1993), and in rat colon membranes, using the NK_2-receptor radioligand [^{3}H]-GR100679 (Hagan et al., 1993). Data were analysed using ALLFIT and affinity (pKi) estimates determined. Results are expressed as mean ± s.e.mean. *In vitro* functional activity was determined in isolated preparations of guinea-pig trachea (GPT) and human bronchus (HuB). Cumulative concentration-effect curves were constructed to the selective NK_1-receptor agonist, substance P methylester (SPOMe), the selective NK_2-receptor agonist, GR64349 (Hagan et al., 1991), or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), in the absence and presence of GR159897. *In vivo* activity of GR159897 (i.v. and p.o.) was tested in anaesthetised guinea-pigs against i.v. GR64349-induced increases in tracheal inflation pressure (TIP), to determine both its potency (dose-ratio $(DR)_{100}$, dose of GR159897 giving 100-fold rightward displacement of GR64349-induced increases in TIP) and duration of action ($t_{1/2}$, time taken for the DR to decrease by 50%).

GR159897 inhibited binding of [^{3}H]-GR100679 to hNK_2-CHO cells (pKi 9.5±0.1; slope 1.1±0.1; n=10) and to rat colon membranes (pKi 10.0± 0.1; slope 1.0±0.1; n=5). GR159897 had negligible affinity for NK_1 (human astrocytoma U373MG) or NK_3 (guinea-pig cerebral cortex) receptor binding sites (pKi 5.7±0.2 and < 5, respectively; n=2). GR159897 (0.1-10μM) caused concentration-related, parallel, rightward displacements of concentration-effect curves to GR64349 in both GPT (pA_2 = 8.7 (95% confidence limits 8.3-9.4), slope = 1.2 (0.8-1.5), n=6) and HuB (pA_2 = 8.6 (8.2-9.6), slope = 0.7 (0.5-1.0), n=2). GR159897 (10μM) had little effect against contractions of GPT to SPOMe or $PGF_{2\alpha}$-induced contractions of GPT or HuB (pK_B <5, n=3-4). *In vivo*, GR159897 (0.04-1.2mg.kg^{-1}, i.v.) caused dose-related antagonism of GR64349-induced increases in TIP, being both potent (DR_{100} = 0.8 (0.6-1.2)mg.kg^{-1}, n=12) and long-acting ($t_{1/2}$ = 92.6±8.1min, n=11). In contrast, GR159897 (4mg.kg^{-1}, i.v.) had no effect against SPOMe-induced increases in TIP (DR < 2, n=2). GR159897 (4mg.kg^{-1}, p.o.) markedly antagonised GR64349-induced increases in TIP (DR: 60min = 51 (40-66, n=8); 120min = 40 (23-71, n=4); 240min = 15 (10-21, n=4), and thus had a duration of at least 4h.

In conclusion, GR159897 is a highly potent non-peptide antagonist at NK_2-receptors, with little activity at NK_1- or NK_3- receptors. At NK_2-receptors *in vivo*, GR159897 has potent and long-lasting antagonist effects following both i.v. and oral administration. GR159897 may be a useful tool for studying the physiological and pathophysiological role of NK_2-receptor activation.

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49P GR159897, A POTENT NON-PEPTIDE TACHYKININ NK_2 RECEPTOR ANTAGONIST, RELEASES SUPPRESSED BEHAVIOURS IN A NOVEL AVERSIVE ENVIRONMENT

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GR159897 ((R)-1-[2-(5-fluoro-1H-indol-3-yl)ethyl]-4-methoxy-4-[(phenylsulfinyl)methyl]-piperidine), is a novel, highly potent, non-peptide tachykinin NK_2 receptor antagonist (Ball et al., 1994). We recently reported the anxiolytic-like effects of peptidic tachykinin NK_2 receptor antagonists in models of anxiety following infusion into the dorsal raphe nucleus (DRN) of the rat (Stratton et al., 1993). We have now investigated the effects of GR159897 in three putative rodent models of anxiety, the mouse light-dark box (Costall et al., 1989), rat social interaction test (File, 1980) and rat elevated plus maze (Handley and Mithani, 1984), following both peripheral (s.c.) and intra-DRN administration. These models measure the suppression of normal behaviours during exposure to a novel aversive environment and can be released by putative anxiolytic drugs. Data were analysed by ANOVA and Dunnett's t-test. Results are presented as mean ± s.e.mean.

In the mouse light-dark box, GR159897 (0.0005-50$\mu g.kg^{-1}$ s.c. in saline, 30min pretreatment (PTT)) increased the time which mice spent in the light compartment during the 5min observation period in a dose-dependent manner. A maximally effective dose (5.0$\mu g.kg^{-1}$) produced a significant ($P<0.05$) 82% increase in time spent in the light with respect to vehicle (vehicle 83±5s, 5$\mu g.kg^{-1}$ 150±9s, n≥9). In the rat social interaction test, under high light unfamiliar conditions, GR159897 (1.25, 12.5 and 125ng intra-DRN in sterile water, pH7.4, 10min PTT) increased interaction time between pairs of rats in a 10min observation period by 83, 110 ($P<0.05$) and 86%, respectively (vehicle 29±3s, 1.25ng 53±9s, 12.5ng 61±11s, 125ng 54±9s, n≥4). On the rat elevated plus maze, GR159897 (1.25, 12.5 and 125ng intra-DRN in sterile water, pH7.4, 10min PTT) produced a dose-related increase in time spent on the open arms in a 3min test period by 41, 105 ($P<0.05$) and 126% ($P<0.05$), respectively (vehicle 30±3s, 1.25ng 42±8s, 12.5ng 61±7s, 125ng 67±7s, n≥5). In all studies, the anxiolytic-like effects of GR159897 were similar to those of diazepam (0.75-1.75$mg.kg^{-1}$ s.c. or i.p.). GR159897 had no effect on locomotor activity and no other overt behaviours were observed.

These results indicate that GR159897, a potent NK_2 receptor antagonist, restores suppressed behaviours in putative rodent models of anxiety following both peripheral and central administration. The DRN is proposed as one possible site of this activity. These findings provide further evidence for an involvement of NK_2 receptors in anxiety-related behaviours.

We would like to thank F.J. Harvey for expert technical assistance.

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50P THE EFFECT OF SUBSTANCE P ON CORTICAL NEURONES *IN VITRO*

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It is possible that substance P (SP) may be involved in Alzheimer's disease (AD). For example, SP-like immunoreactivity is decreased in the cerebral cortex of AD patients (Crystal and Davies, 1982) and some evidence suggests that the cytotoxic effects of β-amyloid can be reversed by SP in rat neocortex (Yankner *et al*, 1990). We have studied the effects of SP in slices of the entorhinal cortex (EC) *in vitro*. The EC has a moderate concentration of SP-containing terminals and binding sites for the peptide (Mantyh *et al*, 1989). It is a site of early neurodegeneration in AD. Experiments were conducted in slices of rat or guinea pig EC maintained at the interface between artificial CSF (at 34°C) and carbogen gas. Intracellular voltage recordings were made from neurones in layers II and V.

The membrane potential (-72.7 ± 0.9mV, mean ± s.e.m.) and input resistance (35.8 ± 6.5mΩ) of around 90% of neurones in layer V of the rat EC (n=64) were unaltered (-71.8 ± 0.8mV, 35.2 ± 6.6mΩ) during perfusion with SP up to 10 μM. Similar results were obtained in layer II (n=49, -70.9 ± 1.2mV, 31.6 ± 4.8mΩ *v* -71.1 ± 1.3mV, 31.6 ± 4.8mΩ). In addition, neither glutamate-mediated excitatory synaptic responses (n=15) nor firing evoked by direct depolarizing current pulses (n=12) were affected by SP. The remaining neurones in either layer showed weak but repeatable, TTX-resistant hyperpolarizing responses (2.6 ± 0.2 mV, n=13, layer II and V pooled) with no detectable change in input resistance, synaptic excitation or directly evoked firing to SP (5-10 μM). There may be a species variation in neurokinin receptors, so we also tested SP on neurones in the EC of the guinea pig. Results were identical to those in rat slices. The general lack of effect was surprising in view of the powerful excitatory actions of SP seen on cortical neurones *in vivo* (Jones and Olpe, 1985). The latter studies were conducted in the anterior cingulate cortex, so we have also tested the effects of SP on neurones in slices of cingulate cortex from both rat and guinea pig. Again, the membrane potential (-84.1 ± 3.2mV) and input resistance (29.7 ± 9.4mΩ) of the vast majority of cells were unaffected by SP up to 10 μM (n=16) with occasional cells slightly hyperpolarized. There was no problem with drug access to the slices since powerful depolarizing and hyperpolarizing effects could be evoked by perfusion with kainic acid (5 μM) and baclofen (10 μM), respectively. Addition of a variety of peptidase inhibitors and an ACE inhibitor (captopril) made no difference to the effectiveness of SP. Finally, we did observe concentration-dependent, reversible, TTX-resistant depolarizing effects of SP (0.05-1.0 μM) on locus coeruleus neurones in slices from rat and guinea pig. In 14 neurones, SP (0.25 μM) evoked a mean depolarization of 4.5 ± 0.3 mV. The responses could be reversibly blocked by the NK_1 receptor antagonist, GR 82334 (5 μM, n=4).

The present results demonstrate a lack of effect of SP on cortical neurones *in vitro* and question whether the excitatory effects observed *in vivo* (Jones and Olpe, 1985) result from direct activation of SP receptors on cortical neurones.

Acknowledgements: We thank Glaxo Group Research for financial support and the gift of peptides.

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51P SPECIFIC AND NON-SPECIFIC EFFECTS OF THE NK_1-RECEPTOR ANTAGONIST CP-99,994 ON LOCOMOTOR ACTIVITY IN GUINEA-PIGS

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There is evidence that NK_1-receptors may modulate the function of the mesolimbic dopamine pathway, both at the level of the cell bodies in the ventral tegmentum (VTA) and in the terminal field of the nucleus accumbens (NAC; Elliott *et al.*, 1992). In guinea-pigs, i.c.v. administration of the NK_1 agonist GR 73632 stimulated locomotor activity. This response was blocked by haloperidol, but surprisingly, not by the nonpeptide NK_1 antagonist CP-96,345 (Mason *et al.*, 1992). We now examine whether locomotor stimulation induced by [Sar^9,$Met(O2)^{11}$]-Substance P (SM-SubP) or dopamine agonists can be antagonised by another NK_1 antagonist, CP-99,994, or its less active enantiomer CP-100,263.

Male guinea-pigs were implanted with bilateral guide cannulae in the lateral ventricles (Isoflurane anaesthesia). A week later the animals were placed into automated locomotor activity cages. Following a one hour habituation period they received haloperidol (0.01-0.5 mg/kg, s.c.) followed 30 min later by infusion of 10 nmol SM-SubP or vehicle into the ventricles. Activity was recorded for one hour. In further experiments, the effects of CP-99,994 (0.3-20 mg/kg, s.c.) and CP-100,263 (0.3-20 mg/kg, s.c.) on activity induced by SM-SubP (10 nmol, i.c.v.), d-amphetamine (1 mg/kg, i.p.) or the D_2-receptor agonist (+)-PHNO (0.1 mg/kg, s.c.) were investigated.

The response to SM-SubP (mean+sem counts: 15.1±1.7) was blocked by a dose of 0.5 mg/kg haloperidol (6.1±1.4). SM-SubP stimulation of activity was also significantly ($p<0.01$) antagonised by the NK_1-receptor antagonist CP-99,994 at 1 (9.2±1.2) and 3 mg/kg (10.1±1.6). However, the same doses of CP-100,263 were ineffective (1 mg/kg: 16.6±2.7; 3 mg/kg: 16.0±2.0). In contrast, the response to amphetamine (30.0±6.1) was reduced by a dose of 10 mg/kg of both isomers (CP-99,994: 14.3±3.7; CP-100,263: 14.7±1.6) and to (+)-PHNO (29.8±4.5) by a dose of 20 mg/kg of each (CP-99,994: 8.7±1.1; CP-100,263: 8.4+0.9).

Antagonism of locomotion induced by the agonist SM-SubP was stereoselective and therefore mediated through NK_1-receptors. Since haloperidol also blocked the effect of SM-SubP, an involvement of dopaminergic systems is implicated in that response. However, locomotor responses to dopamine stimulants could only be antagonised in a non-specific manner. As locomotor stimulation by dopamine agonists is thought to be mediated through the NAC, our findings do not support the proposal that NK_1-receptor modulation of hyperactivity occurs in this nucleus.

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52P CHARACTERISATION OF SUBSTANCE P-INDUCED EOSINOPHIL ACCUMULATION AND OEDEMA FORMATION IN GUINEA-PIG SKIN

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The neuropeptide substance P (SP), released from peripheral sensory nerve endings, is a major mediator of neurogenic inflammation and has been shown to induce granulocyte infiltration in mouse skin via a mast cell dependent mechanism (Matsuda *et al.*, 1989). The aim of this study was to investigate the mechanisms of SP-induced eosinophil accumulation and oedema formation in guinea-pig skin. ^{111}In-eosinophil accumulation and ^{125}I-albumin leakage (μl plasma) were measured in guinea-pig skin as previously described (Faccioli *et al.*, 1991). Using a 2h accumulation period, intradermal (i.d.) SP caused a dose-dependent ^{111}In-eosinophil accumulation and oedema formation with significant cell accumulation occurring at 10^{-10} moles/site (2241±572 cells/site compared to 784±200 i.d. saline $p<0.01$ $n=10$) and significant plasma protein leakage occurring at 10^{-13} moles/site (16.8±0.8μl compared to 11.8±0.8μl i.d. saline $p<0.01$ $n=11$). Maximal levels of both eosinophils and albumin were detected in the skin 1h after injection of SP and remained elevated for 4h.

Cell accumulation induced by SP (10^{-9} moles/site) was not significantly inhibited by the NK_1 receptor antagonists RP 67580 (Garret *et al.*, 1991; 10nmole/site co-injected i.d. with SP) or CP 96345 (Snider *et al.*, 1991; 1mg/kg i.v.). However, the oedema formation at lower doses of SP was significantly inhibited by both antagonists (e.g. 10^{-11}moles/site SP elicited 37.6±2.8μl plasma compared to 19.9±5.6μl in the presence of RP 67580 and 17.9±2.2μl with CP 96345 $p<0.01$ $n=6$-10). The analogue CP 96344, which is without activity on NK_1 receptors, had no inhibitory activity. In order to investigate the roles of endogenous histamine and PAF in the SP elicited responses, the effects of their respective antagonists chlorpheniramine (10μg/site) and UK 74505 (Cooper *et al.*, 1990; 2.5mg/kg i.v.) were studied. Neither antagonist significantly inhibited cell accumulation or oedema formation.

Intravenous administration of an anti-CD18 monoclonal antibody, 6.5E $F(ab')_2$ (Andrew *et al.*, 1993; 2.5mg/kg), effectively inhibited SP (10^{-9} moles/site)-induced eosinophil accumulation (6797±2231 cells/site reduced to 988±172 in the presence of the antibody, with no effect on numbers of circulating cells $p<0.01$ $n=6$) but had no effect on oedema formation.

These results suggest that SP may act through two independent mechanisms, a direct NK_1 receptor-dependent event to cause oedema formation and an NK_1 receptor-independent event, possibly via mast cell degranulation, to induce eosinophil accumulation.

This work supported by The Wellcome Trust and The National Asthma Campaign.

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53P AGONIST ACTIVITY AND SELECTIVITY OF [Phe^{36}]-NEUROPEPTIDE Y(13-16) IN ANAESTHETISED, AREFLEXIC RATS

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Neuropeptide Y (NPY) has effects on the cardiovascular system via putative post-synaptic (Y_1) and presynaptic (Y_2) receptors. An analogue of NPY, NPY(13-36) has been reported to be a selective agonist at the Y_2 receptor (Sheikh *et al*, 1989). The agonist activity and selectivity *in vivo* of NPY(13-36) has been compared with a novel analogue in which the C-terminal tyrosine amide has been replaced by phenylalanine amide, [Phe^{36}]-NPY(13-36).

Non-recovery experiments were carried out in pentobarbitone-anaesthetised, bilaterally-vagotomised, AP strain rats which had been catecholamine-depleted with syrosingopine and in which the mast cell degranulating effects of the peptides were minimised as described previously (Haworth *et al*, 1992). Increase in systolic blood pressure was measured as an index of Y_1 receptor agonism and inhibition of the increased pulse interval following electrical stimulation of the right vagus nerve as an index of Y_2 receptor agonism (Haworth & Thomson, 1993). [Phe^{36}]-NPY(13-36) inhibited vagally-mediated bradycardia. Its ED_{50}, 64.4±12.5 nmol kg^{-1} i.v. (mean±s.e.mean, n=3) was similar to that of NPY(13-36), 54.4±12.9 nmol kg^{-1} (n=4) but was 8.5-fold greater than that reported previously for NPY (Haworth & Thomson, 1993). [Phe^{36}]-NPY(13-36) was a poor pressor peptide, increasing blood pressure by only 17±5 mmHg (n=3), approximately 20% of the previously reported maximum response to NPY (Haworth & Thomson, 1993), at a dose close to its Y_2 ED_{50} (79 nmol kg^{-1}), by 30±11 mmHg at 240 nmol kg^{-1} and by 46±16 mmHg at the highest dose tested (790 nmol kg^{-1}). NPY(13-36) was a slightly more potent pressor peptide than [Phe^{36}]-NPY(13-36), increasing blood pressure by 27±2 mmHg (n=4) at 70 nmol kg^{-1} and 41±4 mmHg at 211 nmol kg^{-1}, the highest dose tested . Vagal inhibition by [Phe^{36}]-NPY(13-36) was slow in onset and long lasting. After injecting 400 nmol kg^{-1}, maximal inhibition (82.3±1.8%, n=3) was not reached until 10 min post-injection and had declined only slightly by 30 min. In contrast, the increase in systolic blood pressure was maximal (42±13 mmHg) at 1.5 min post-injection but had declined to 15±8 mmHg by 10 min and 5±3 mmHg at 30 min.

These results indicate that [Phe^{36}]-NPY(13-36) has similar Y_2 agonist potency to NPY(13-36) and slightly improved selectivity *in vivo*. [Phe^{36}]-NPY(13-36) has long lasting Y_2 agonism and may be a useful alternative to NPY(13-36) when investigating the receptor subtype(s) responsible for the effects of NPY *in vivo*.

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54P HUMAN α-CALCITONIN GENE-RELATED PEPTIDE (hα-CGRP) CAUSES AN ENDOTHELIUM-DEPENDENT VASORELAXATION IN THE RAT ISOLATED RENAL AND PULMONARY ARTERY

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CGRP is a potent vasodilator in many vascular beds in various species. In the rat thoracic aorta, *in vitro*, CGRP has been demonstrated to act via an endothelium-dependent pathway (Gray & Marshall, 1992). An endothelium-independent mechanism has been described in rat mesenteric resistance arterioles (Marshall *et al*,1988). The aim of these studies was to examine the mechanism of relaxation by hα-CGRP in the rat isolated renal and pulmonary arteries.

Renal and pulmonary arteries were removed from male Sprague Dawley rats (350-450g). Endothelium-denuded (gentle abrasion of the intima) and -intact rings were mounted on wires in Krebs solution, bubbled with 95% O_2/ 5% CO_2, maintained at 37°C and placed under 0.5g resting tension. The effect of cumulatively administered hα-CGRP (10^{-11}-10^{-6}M), acetylcholine (10^{-10}-10^{-6}M) and sodium nitroprusside (10^{-9}-10^{-6}M) was measured on submaximal contractions to noradrenaline ($3x10^{-7}$M) or phenylephrine ($3x10^{-8}$M) in the rat renal and pulmonary arteries respectively.

Hα-CGRP induced a dose dependent vasorelaxation (maximum over 90% of spasmogen-induced tone) with a pD_2 of 8.7±0.1 (mean± s.e.mean) and 8.6±0.1 in the renal and pulmonary arteries respectively. Removal of the endothelium or pretreatment (for 20 min.) with the nitric oxide synthase inhibitor, N^G-nitro-L-arginine (L-NOARG, 10^{-5}M), abolished the acetylcholine and hα-CGRP relaxations in both preparations whereas those to sodium nitroprusside were unaltered (pD_2 7.9±0.1 in the renal and pulmonary arteries). The inhibitory effect of L-NOARG (10^{-5}M) on hα-CGRP responses was reversed by L-arginine (10^{-4}M) but not by D-arginine (10^{-4}M). Calmidazolium (30 min.;10^{-5}M), an endothelium-dependent calmodulin inhibitor (Weinheimer & Osswald, 1986), abolished hα-CGRP and acetylcholine responses without modifying relaxations to sodium nitroprusside in both the renal and pulmonary arteries.

The results demonstrate that in the rat renal and pulmonary arteries, hα-CGRP causes vasorelaxation via an endothelium-dependent pathway. The inhibitory effect of L-NOARG (reversed stereospecifically by L-arginine) suggests the involvement of nitric oxide, derived from L-arginine via nitric oxide synthase. This enzyme is Ca^{2+} and calmodulin dependent (Förstermann *et al*,1991). The calmodulin inhibitor, calmidazolium, blocked endothelial-dependent relaxations but not those to sodium nitroprusside in the arterial rings. Therefore, CGRP responses in the renal and pulmonary arteries may be mediated via calcium-dependent activation of the nitric oxide synthase in the endothelium.

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55P α_{1A}- AND α_{1B}-ADRENOCEPTOR SUBTYPES AND CONTRACTIONS OF THE RAT THORACIC AORTA TO NORADRENALINE, PHENYLEPHRINE AND OXYMETAZOLINE

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We have previously shown that the α_1--adrenoceptor(s) responsible for the noradrenaline (NA)-induced contractions of the rat thoracic aorta are sensitive to both α_{1A}-subtype selective antagonists (WB-4101 and abanoquil) and the α_{1B}-selective alkylating agent chlorethylclonidine (CEC) (Wenham & Marshall., 1992). This data, together with that of others (Aboud *et al.*, 1993), has raised the question of whether these contractions in the aorta are mediated via both the α_{1A}- and the α_{1B}-adrenoceptors or whether a single putative receptor sharing the characteristics of both of the subtypes is responsible. The aim of this current study was to compare the effects of abanoquil and CEC on NA induced contractions with those induced by phenylephrine (PE) and oxymetazoline (OXY).

Thoracic aortic rings (minus endothelium), 4-5mm long, from male Sprague Dawley rats (300-400g) were mounted in organ baths containing Krebs buffer at 37°C under oxygenation with 95%/5% O_2/CO_2 for the recording of isometric tension. Cumulative concentration-response curves were performed to either NA, PE or OXY before and after 30 min equilibrations with antagonist. In the case of CEC, a 30 min washout period followed thé eqilibration. In some experiments PE or OXY concentration-response curves were performed (in the absence of antagonist) following an initial NA curve. Data are given as the mean±s.e.mean of at least 4 separate experiments.

The three agonists used in this study contracted the aortic rings in a concentration dependent manner with pD_2 values of 8.4±0.1, 8.0±0.1 and 6.7±0.1 for NA, PE and OXY respectively. PE and OXY acted as partial agonists with respect to NA causing 86.5±4.3% and 77.2±5.2% respectively of the maximum obtained in the first NA curve. Prazosin antagonised all three agonists consistent with α_1-adrenoceptor mediated responses with a pA_2 value against NA of 10.4 (slope=1.1±0.3) and 'relative pK_B' values (due to a 20% reduction in the maximum with prazosin) against PE and OXY of 10.3±0.1 and 9.4±0.2 respectively. Abanoquil acted non-competitively and at 1nM and 10nM concentrations caused a reduction in the maximal response to NA-induced contractions of 28.4±8.0 and 86.1±4.3% respectively. Contractions to OXY were shifted 100-fold to the right with a 57.9±9.0% reduction in the maximum by abanoquil (1nM) and contractions to PE were almost completely abolished. CEC (10μM) caused a large bi-phasic (second phase beginning at [NA]>10μM) inhibition of NA-induced contractions of which only the first phase was sensitive to antagonism by prazosin (100nM) i.e the second phase may be mediated via a non-α_1-adrenoceptor. CEC (10μM) caused an 82.0±7.3% reduction in the maximum obtained to PE but responses to OXY were not greatly altered (shifted 2-fold to the right with a 14.5±3.2% reduction in the maximum).

Contractions to OXY were antagonised by the α_{1A}-selective compound abanoquil but were insensitive to the α_{1B}-selective compound CEC, demonstrating that OXY acted through the α_{1A}-subtype. Contractions to NA and PE were sensitive to both antagonists showing that, in addition to the α_{1A}-mediated response, there was an α_{1B} component to contractions. The results from this study are consistent with the hypothesis that α_1-adrenoceptor agonists can contract the rat thoracic aorta via α_{1A}- and α_{1B}- adrenoceptors and that it is unnecessary to postulate a novel α_1-adrenoceptor subtype.

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56P THE EFFECTS OF NIFLUMIC ACID ON NORADRENALINE-INDUCED CHLORIDE CURRENTS AND MECHANICAL RESPONSES OF THE RAT PORTAL VEIN

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It has been previously reported that noradrenaline (NA) activates a chloride (Cl) conductance in rabbit and rat portal vein (Byrne & Large, 1988; Pacaud et al., 1989b), a mechanism by which agonists could depolarise the smooth muscle and activate voltage-operated calcium (Ca) channels. Since niflumic acid (NFA) is a potent inhibitor of Ca-activated Cl currents in rat portal vein (Pacaud et al., 1989a), we decided to study whether NFA could reduce NA-elicited Cl currents in rat portal vein cells and if any such inhibition was reflected by a reduction in NA-induced contractile responses of the whole tissue.

Rat portal vein cells were dissociated using a collagenase/pronase enzyme treatment and were then superfused with a potassium(K)-free (isosmotically replaced with caesium) physiological salt solution. Whole-cell currents were recorded with the 'perforated-patch' configuration of the patch-clamp technique, using amphotericin B (pipette concentration of 300 μg/ml). NA (10 μM) was applied by pressure ejection. At a holding potential of -60 mV, application of NA to cells generated an inward current, the reversal potential (E_{rev}) of which was close to the Nernstian-derived chloride equilibrium potential (E_{Cl}) value of -2 mV. When some of the external Cl was replaced with isethionate, the E_{rev} of the current was significantly shifted in the positive direction ($p<0.05$; Students' t-test) to a value close to the altered E_{Cl} (+20 mV). However, when some of the external sodium was replaced with Tris, the E_{rev} of the current was not significantly changed ($p>0.05$). At -60 mV, NFA (10 μM) produced 68±12% inhibition of the NA-induced inward current. In experiments in K-containing solutions at a holding potential of -2 mV (the E_{Cl}) NFA (10 μM) produced no effect on NA-evoked outward K currents. Cumulative concentration effect experiments to NA and KCl (4-6 min contact time) were conducted in the absence and presence of 10 μM NFA in whole rat portal veins. Incubation with 10 μM NFA (30 min) produced a significant reduction (25.7±1.6%: $p<0.001$) in the maximal obtainable response but no significant change in the NA EC_{50} value ($p>0.05$). However, NFA (10 μM) also produced a marked reduction (43.4±2.2%: $p<0.001$) in the maximal obtainable response to KCl with no significant effect on the EC_{50} value for this spasmogen ($p>0.05$). All values represent mean ± sem, n=3-6.

The results of the present study indicate that NFA inhibits NA-induced Cl currents in rat portal vein. This action may contribute to the observed inhibition of NA-evoked mechanical activity. However, at the same concentration, NFA also significantly attenuated KCl-induced increases in tension, which suggests that the vasorelaxant effect of NFA may involve additional mechanisms.

Supported by the Wellcome Trust.

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57P THE EFFECT OF 1,4-DIHYDROPYRIDINES ON α_1-ADRENOCEPTOR AGONIST- AND DEPOLARISATION-INDUCED CONTRACTIONS OF PERFUSED RESISTANCE ARTERIES

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The present study was undertaken to compare two newer dihydropyridine calcium antagonists (manidipine (MAN) and nisoldipine (NIS)) with the classic prototype of this group; nifedipine (NIF). The experiments were performed in perfused (5ml/min; initial perfusion pressure 18.1±2.4mmHg, n=24) rat mesenteric vascular bed preparations, which were cut from the intestine. After equilibration for 20 min the vascular preparations were subjected for 2 min to a depolarizing physiological salt solution (PSS) containing 40mM K^+, thus causing a contraction. With 20 min intervals this was followed by a 2 min contraction induced by methoxamine (3.0μM) and five subsequent contractions by the high K^+ PSS. Pretreatment of the drugs (MAN; 1.0-3.0-10.0nM, NIS; 1.0-3.0-10.0nM, NIF; 1.0-10.0-100.0nM) was started after the last K^+-induced contraction. With 60 min intervals the preparations were contracted six times by means of high K^+ PSS for 2 min. Twenty min after the last K^+-induced contraction a cumulative concentration-response curve (CRC) for methoxamine (0.1-10.0μM) was constructed. CRC-parameters were calculated by a computer program based on a Hill-relationship.

MAN was the most potent inhibitor of the high K^+ PSS-induced contractions (Emax=100.0±1.02%, pIC50=8.45±0.18) followed by NIS (Emax=96.5±1.23%, pIC50=8.37±0.01) and NIF (Emax=85.2±0.61%, pIC50=8.48±0.01). The CRC parameters for methoxamine in untreated preparations amounted to Emax=207±10mmHg and pEC50=5.36±0.01, respectively. The calcium antagonists concentration-dependently decreased the methoxamine-induced contractions (NIF: Emax=107.9±16.4mmHg, pIC50=8.96±0.13, n=6; NIS: Emax=109.6±13.8mmHg, pIC50=8.71±0.11, n=6 and MAN: Emax=87.4±1.8mmHg, pIC50=8.83±0.04 n=6). The calcium antagonists used in the present study did not influence the EC50 of methoxamine-CRC.

In conclusion: all 1,4-dihydropyridines investigated inhibited the α_1-adrenoceptor- and depolarization-induced contractions of the rat isolated perfused mesenteric vascular bed preparation in a concentration-dependent manner. The contractions induced by depolarization were inhibited more potently and effectively when compared to those induced by methoxamine. This finding indicates the involvement of an L-type calcium-independent effector pathway subsequent to α_1-adrenoceptor stimulation in the preparation used in this study.

58P THE ROLE OF α_1-ADRENOCEPTOR SUBTYPES IN THE REGULATION OF RENAL TUBULAR PROCESSES IN THE 2K1C GOLDBLATT HYPERTENSIVE RAT

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The kidney possesses a dense sympathetic nervous innervation and both α_1 and α_2 adrenoceptors have been shown to be present in the renal cortex. Recent studies showed that the α_{1a} subtype is predominantly involved in the regulation of renal haemodynamics in the 2K1C Goldblatt hypertensive rats (Munavvar and Johns, 1992). The aim of this study was to determine which subtypes of the α_1-adrenoceptors were involved in regulating renal sodium and water handling in experimental hypertension. Male 2K1C Goldblatt hypertensive rats, 300-325g, were anaesthetised (sodium pentobarbital, 60 mg kg^{-1} i.p.). After tracheotomy, a carotid artery and jugular vein were cannulated for the measurement of blood pressure and infusion of saline. The left kidney was exposed and an electromagnetic flowmeter probe was fitted to the left renal artery for determination of renal blood flow. The iliac artery was cannulated for close renal arterial infusion of all drugs and vehicle (1% inulin in 150 mmol NaCl). Following completion of surgery, 2ml of vehicle was given i.v. and infusion at a rate of 6 ml h^{-1}commenced. The animals were given 2 hours to stabilise. Each experiment consisted of five 15 min. clearance periods, two before and two after a period during which phenylephrine 100 μg kg^{-1} h^{-1} was infused. After the first set of five clearance collections, a second set was taken in the presence of chloroethylclonidine (CEC), an α_{1b}- alkylating agent, or 5 methylurapidil (5Me-U), an α_{1a}- antagonist, both at concentrations of 10 μg kg^{-1} h^{-1}

GROUP	VEHICLE		CEC		VEHICLE		5Me-U	
	CONTROL	EXPT	CONTROL	EXPT	CONTROL	EXPT	CONTROL	EXPT
RBF (ml kg^{-1} min^{-1})	20.4±0.7	18.8±0.8	19.3±0.7	18.6±0.7	18.4±0.5	16.2±0.5	17.9±0.3	17.2±0.5
GFR (ml kg^{-1} min^{-1})	3.1±0.1	3.0±0.1	2.4±0.2	2.5±0.3	3.9±0.4	3.8±0.4	2.9±0.5	3.0±0.5
UV (μl kg^{-1} min^{-1})	134.2±5.8	46.1±7.5*	130.0±5.4	55.6±10.4*	73.2±7.0	38.6±6.5*	61.3±14.1	63.9±12.4
$U_{Na}V$ (μmol kg^{-1} min^{-1})	26.8±1.7	8.3±1.9*	26.1±1.5	10.6±2.3*	13.4±1.8	4.7±0.9*	10.8±3.3	11.4±3.0
FE_{Na} (%)	6.1±0.5	1.9±0.4*	8.1±1.2	3.4±1.2*	2.6±0.3	0.9±0.1*	2.6±0.5	2.6±0.4
MAP (mmHg)	149±4	161±5	139±5	148±2	148±2	163±5	139±2	145±2
RPP (mmHg)	145±3	147±3	140±4	139±4	153±2	153±2	146±2	147±3

RBF-renal blood flow, UV- urine flow, $U_{Na}V$- absolute sodium excretion, FE_{Na} -fractional sodium excretion, MAP- mean arterial pressure, RPP- renal perfusion pressure, Control- average value of the clearances before and after phenylephrine infusion, EXPT- clearance values during phenylephrine infusion. *- P<0.05 vs. control, (ANOVA), n=5.

The phenylephrine induced antinatriuresis and antidiuresis in the absence of renal haemodynamic changes probably reflect an action at the level of the epithelial cells.The observation that the tubular responses were blocked by 5Me-U at 10 μg kg^{-1} h^{-1} but not CEC at 10 μg kg^{-1} h^{-1} would suggest that they might be mediated by α_{1a}- adrenoceptors.

Munavvar, A.S. & Johns, E.J. (1992) Br. J. Pharmacol., 107, 326P.

59P COMPARISON OF DRUG AFFINITIES AT CLONED AND RAT TISSUE α_1-ADRENOCEPTOR SUBTYPES

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We have determined the affinities at cloned α_1-adrenoceptor subtypes for 5-methylurapidil, methoxamine, noradrenaline, oxymetazoline, phentolamine, SDZ NVI-085 ((-)-(4aR,10aR)-3,4,4a,5,10,10a-hexahydro-6-methoxy-4-methyl-9-methylthio-2H-naphth[2,3,b]-1,4-oxazine HCl), and (+)- and (-)-tamsulosin. The constructs pCMVα_{1A} (rat $\alpha_{1A/D}$), pcDV1Rα_{1B} (rat α_{1B}), and pBCα_{1C} (bovine α_{1C}) containing the entire coding region of the indicated subtypes were transiently transfected into COS cells as described (Schwinn et al. 1990; Lomasney et al. 1991). Binding of [^{3}H]prazosin to α_1-adrenoceptors in membranes from transfected cells was performed as described (Michel et al. 1993). Data are mean ± s.e. mean of 3-4 experiments.

Table 1: Drug affinities (-log K_i) at cloned subtypes

	5-methyl-urapidil	methox-amine	norad-renaline	oxymeta-zoline	phentol-amine	SDZ NVI-085	(-)-tam-sulosin	(+)-tam-sulosin
α1A/D	6.2±0.1	4.9±0.1	7.4±0.1	5.9±0.0	7.8±0.0	5.7±0.0	10.1±0.1	7.9±0.1
α1B	6.9±0.1	3.8±0.1	6.0±0.1	6.5±0.1	7.7±0.1	4.9±0.0	9.1±0.2	7.0±0.2
α1C	8.5±0.4	5.5±0.1	6.5±0.1	7.9±0.1	8.7±0.1	6.5±0.1	10.6±0.3	9.5±0.4

Thus, all drugs tested except noradrenaline had similar affinities at $\alpha_{1A/D}$- and α_{1B}-adrenoceptors indicating that these two subtypes may have a rather similar pharmacological profile. When affinities at cloned subtypes were compared with those in rat tissues determined with identical methods (Michel et al. 1993), drug affinities at splenic α_{1B}-adrenoceptors and at the low affinity sites in cerebral cortex and kidney were matched best by those at the cloned α_{1B}-adrenoceptor. Drug affinities at the high affinity site in rat cerebral cortex and kidney (pharmacologically defined α_{1A}-adrenoceptor) were matched best by those at the cloned α_{1C}-adrenoceptor. Since the cloned α_{1C}- in contrast to the pharmacologically defined α_{1A}-adrenoceptor is sensitive to chloroethylclonidine (Schwinn et al. 1990), we conclude that it is similar but not identical to the α_{1A}-adrenoceptor.

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60P PHARMACOLOGICAL CHARACTERISATION AND TISSUE DISTRIBUTION OF HUMAN α_2-ADRENOCEPTOR SUBTYPES

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α_2-Adrenoceptor (AR) heterogeneity has been confirmed by molecular biological techniques in that three cDNAs encoding structurally-distinct human α_2-ARs have been cloned and shown to reside on different chromosomes (α_2-C10, α_2-C4 and α_2-C2; Lomasney *et al.*, 1990). For the present study, we have performed a detailed pharmacological characterization of these three gene products expressed in transfected chinese hamster ovary (CHO) cell-lines using [^{3}H]-rauwolscine as a probe. In addition, we report upon the mRNA distribution of the α_2-C10 and α_2-C4-AR subtypes in a number of human tissues.

Three different transfected CHO cell-lines which express either α_2-C10, α_2-C4 or α_2-C2-ARs were grown in monolayer culture. [^{3}H]-Rauwolscine binding to cell membrane preparations was then performed at 25°C and 10 µM phentolamine was used to define non-specific binding. The potency of a number of drugs to compete with [^{3}H]-rauwolscine for these α_2-ARs was determined. The three α_2-ARs showed differential affinities for oxymetazoline (pK_i values of 8.58, 6.64 and 5.87 for α_2-C10, α_2-C4 and α_2-C2-ARs, respectively). A highly significant correlation (r=0.925) was obtained upon comparing the pK_i values of 14 drugs including yohimbine, oxymetazoline, idazoxan, chlorpromazine, spiroxatrine, WB4101 and prazosin to inhibit [^{3}H]-rauwolscine binding to the α_{2A}-AR cell-line HT29 and the α_2-C10-AR cells. A similar analysis of the pharmacological profile of [^{3}H]-rauwolscine binding to the α_{2B}-AR subtype of rat neonatal lung, on the other hand, exhibited a good correlation (r=0.916) with the α_2-C2-AR cells. The pK_i values obtained with the α_2-C4-AR cells were best correlated (r=0.859) with those of the α_{2C}-AR subtype of the OK cell-line. These results therefore provide evidence that the α_2-C10 gene product exhibits the pharmacological profile of an α_{2A}-AR, whereas the α_2-C2 and α_2-C4 gene products correspond to species homologues of the α_{2B}- and α_{2C}-AR subtypes, respectively.

Poly(A)$^+$ RNAs from human heart, brain, placenta, liver, lung, skeletal muscle, kidney and pancreas were hybridized using the Northern Blot technique with α_2-C10 and α_2-C4-AR DNA probes. A 4.1 Kb mRNA species was detected in all tissues with the α_2-C10 probe and was particularly abundant in the pancreas. The α_2-C4 probe detected two species of 2.9 and 2.4 Kbs. The 2.9 Kb species was detected in all tissues and the 2.4 Kb species in all tissues except liver and lung. The 2.9 and 2.4 Kb bands were particularly abundant in kidney and pancreas. Our data suggest the presence of two α_2-C4 (or α_{2C}) mRNAs in man and illustrate the differential tissue distribution patterns of α_2-AR subtypes.

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61P A STIMULATORY ACTION OF CHLOROETHYLCLONIDINE IN THE RESPONSE OF THE RAT VAS DEFERENS TO SINGLE PULSE FIELD STIMULATION (SPFS)

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We have reported (Mallard *et al*, 1992) that CEC abolished the NA component of the response to SPFS without affecting either the purinergic component or the response to exogenous NA. Bultmann & Starke (1993) have reported that CEC has prejunctional α_2-adrenoceptor agonist activity and have questioned the suitability of CEC as a tool in the characterisation of α-adrenoceptors. The present experiments were undertaken to further evaluate the action of CEC on rat vas deferens.

Whole or prostatic and epididymal thirds of rat vas were suspended in 7ml tissue baths containing Krebs solution and stimulated with single square wave pulses (width 0.8ms) at 5 minute intervals. A computer system controlled stimulus delivery and collected on-line digitised (100Hz) tension data.

CEC (0.1-3uM) selectively inhibited the NA component of the response to SPFS in a concentration related manner (to 36.6 ± 2.9% of control at 3μM) with much less effect (to 67.2±3.6% of control at 3μM) on the putative purinergic component as previously reported (Mallard et al,1990). In the presence of prazosin (300nM) the purinergic component was inhibited to a much greater extent by CEC (27.6±2.6% of pre-prazosin control at 3μM). Investigations using epididymal and prostatic portions revealed that CEC (0.1uM-3uM) had modest inhibitory effect (to 67.4±6.4% of control) on the response in the prostatic portion but inhibited to 49.3±9.1% of control the epididymal response which peaked at 710±0.01ms after the stimulus. In the epididymal portion 1 & 3μM CEC elicited a new component which peaked at 433±0.01ms after the stimulus. The development of this new component was prevented by nifedipine (10uM), prazosin (300nM) or suramin (1mM). When the responses from prostatic and epididymal segments in the presence of CEC were summated the time course of the composite response was indistinguishable from that obtained from a whole vas.

Our results demonstrate that CEC produces a hitherto unreported early component peaking at 433ms after the stimulus in the response of the epididymal portion of the rat vas to SPFS which masks the presynaptic inhibitory action of CEC on the purinergic component in whole vas responses. CEC did not produce a direct contractile effect on smooth muscle in the absence of electrical stimulation suggesting that this new peak is dependent on the neurotransmission process and appears to require some contribution from activation of both P_{2X}-purinoceptors and $\alpha_{1(?A)}$-adrenoceptors.

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62P β_2-ADRENOCEPTORS MEDIATE INHIBITION OF THROMBIN-STIMULATED INCREASES IN ENDOTHELIAL CELL MONOLAYER PERMEABILITY

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We have investigated the β-adrenoceptors modulating albumin clearance across endothelial cell monolayers *in vitro* using the β-adrenoceptor agonists, isoprenaline (non-selective), RO-363 (β_1-selective), salbutamol (β_2-selective) and CL-316,243 (β_3-selective), and antagonist, ICI-118,551 (β_2-selective).

Changes in albumin clearance across bovine pulmonary artery endothelium (CPAE, obtained from ECACC) were measured as previously described (Allen & Coleman, 1993). Clearance levels quoted are differences between 1 hour test and basal periods (mean±s.e.mean, basal period clearance=11.4±0.2μl.hr^{-1}, n=422). Thrombin (10U.ml^{-1}) was used to stimulate increases in albumin clearance. Isoprenaline (1nM-1μM) and salbutamol (10nM-10μM) caused concentration-related inhibition of thrombin-stimulated albumin clearance. Isoprenaline (EC_{50} 24nM, n=7) and salbutamol (EC_{50} 19nM, n=7) were approximately equipotent but isoprenaline (1μM) inhibited thrombin-stimulated albumin clearance by a maximum of 55% (control=1.7±0.9μl.hr^{-1}, thrombin=13.6±0.5μl.hr^{-1}, thrombin + isoprenaline=7.1±1.0μl.hr^{-1}, n=7), whereas salbutamol (1μM) reduced thrombin-stimulated clearance to below control levels (control=1.3±0.4μl.hr^{-1}, thrombin=5.8±1.9μl.hr^{-1}, thrombin + 1μM salbutamol=-0.1±0.33μl.hr^{-1}, n=7). When compared within the same experiment, maximally effective concentrations (1μM) of isoprenaline and salbutamol caused 49% and 81% inhibition of thrombin-stimulated albumin clearance respectively (control=4.2±0.8μl.hr^{-1}, thrombin=13.2±2.0μl.hr^{-1}, thrombin + isoprenaline=8.8±1.4μl.hr^{-1}, thrombin + salbutamol=5.9±1.8μl.hr^{-1}, n=6), however these values were not significantly different ($p>0.05$). Neither RO-363 (10μM) nor CL-316,243 (10μM) had any significant effect on thrombin-stimulated albumin clearance (n=6, $p>0.05$ vs. thrombin alone). Both isoprenaline (1μM) and salbutamol (1μM) also caused small, but significant ($p<0.05$) reductions in albumin clearance across unstimulated monolayers (control=2.1±0.5μl.hr^{-1}, isoprenaline=-1.2±0.2μl.hr^{-1}, salbutamol=-0.5±0.7μl.hr^{-1}). In contrast, neither RO-363 (1μM) nor CL-316,243 (1μM) had any significant ($p>0.05$) effect on albumin clearance across unstimulated monolayers. The inhibition of thrombin-stimulated albumin clearance by isoprenaline was antagonised by ICI-118,551 (0.1&1μM, n=6), producing rightward shifts of the concentration-effect curves for isoprenaline (pK_B 8.3) with no significant ($p>0.05$) alteration of the maximal response. ICI-118,551 (1μM) had no direct effect on either unstimulated or thrombin-stimulated albumin clearance ($p>0.05$, n=6).

These results demonstrate that β_2-adrenoceptors are present on CPAE cells, and that these receptors mediate inhibition of thrombin-stimulated increases in permeability to albumin. There appears to be no role for either β_1- or β_3-adrenoceptors in the modulation of CPAE permeability. The possible difference in maximal inhibition between isoprenaline and salbutamol warrants further investigation.

Allen, M.J. & Coleman, R.A. (1993). This meeting.

63P EFFECTS OF A PHOTOSENSITIVE NITRIC OXIDE DONOR ON ISOLATED GUINEA-PIG TAENIA COLI

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Iron-sulphur cluster nitrosyl compounds accumulate in endothelial cells and can produce a vasodilator action (Flitney *et al*, 1992). In the experiments reported here we have found that the cluster nitrosyl compound heptanitrosyl-tri-μ3-thioxotetraferrate(1-), also known as Roussin's Black Salt (RBS), has little or no effect on the contraction of the smooth muscle of the guinea-pig taenia coli in the absence of light but causes a marked relaxation when photon-activated. Small lengths (1-2cm) of isolated taenia coli were superfused at 32°C and muscle activity recorded isotonically in a light-sealed enclosure. After exposure to RBS (50μM) for 30min the nitrosyl compound was washed out and a sustained contraction produced with carbachol (50μM) or KCl (23.5mM). No relaxation was seen in the absence of light but photon-irradiation of the RBS-treated muscle via fibre optic probes from a quartz-halogen light source elicited a prompt relaxation of the precontracted muscle, the magnitude of which depended upon the intensity ($1.1x10^3$ to $1.1x10^5$lux), duration (30sec to 20min) and wavelength (400 to 800nm) of the incident illumination. Three minute periods of illumination at 11,000 lux caused a reversible and biphasic inhibition of both carbachol and KCl-evoked tone. These relaxations coincided with the duration of illumination and were reproducible at 10min intervals, often for several hours, with a maximal relaxation amounting to >90% that of the tone produced by carbachol (50μM).

The nitric oxide synthase inhibitor N^G-nitro-L-arginine (60μM) caused no significant inhibition of the photon-induced relaxation of RBS-treated muscle, ie., from control values of 91±4.5% (n=4) to 86±2.1% (n=4). In contrast, N-methylhydroxylamine (2mM), L-cysteine (10mM), DL-dithiothreitol (2mM), methylene blue (30μM) and haemoglobin (20μM) all inhibited significantly ($P<0.001$) the relaxation response, ie., from 91±4.5% (n=4) to 34±3.4% (n=4), 10±1.1% (n=5), 12±1.7% (n=4), 26±3.3% (n=7) and 21+3.6% (n=5), respectively, that of the sustained response to carbachol (50μM). These inhibitory effects on the RBS-induced relaxation response were reversible but methylene blue produced the most prolonged action.

Although further experiments are required to fully resolve the molecular mechanism of action of the nitrosyl cluster compounds, it is concluded that sequestered or bound RBS when photon-activated, even at low light intensities, liberates NO which rapidly relaxes smooth muscle cells probably via cGMP-dependent pathways.

We are grateful to Dr. A. R. Butler for the supply of RBS.

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64P BOTH ATP AND NITRIC OXIDE (NO) MAY BE INVOLVED IN THE NEURONALLY-MEDIATED RELAXATION OF THE GUINEA-PIG INTERNAL ANAL SPHINCTER (gpIAS)

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Field stimulation of circular muscle of the gpIAS causes membrane hyperpolarization and relaxation (Lim & Muir,1983; Rae & Muir, 1993). The electrical characteristics of the transmitters involved (Rae & Muir, 1993) and their contribution to relaxation have now been examined using conventional simultaneous microelectrode and mechanical recording techniques. Field stimulation (1-5 pulses, 1-40 Hz, 0.1ms, supramaximal voltage) of the gpIAS (resting membrane potential -42.9 ± 4.3 mv, n = 306) in the presence of atropine and phentolamine (each 1μM) produced tetrodotoxin - sensitive inhibitory junction potentials (ijps). Each ijp consisted of a fast, frequency-dependent , apamin (0.3μM) - sensitive component (maximum amplitude 40mV, time to reach nadir 338 ± 21ms, n = 26, following a single pulse). In the presence of apamin (0.3μM) a significantly ($p<0.05$) slower and smaller (maximum amplitude 16.9mV, time to reach nadir 671±114ms, n = 16, following a single pulse) ijp component was unmasked. This apamin-insensitive component was abolished by L-NAME (but not D-NAME, each 0.1mM), which inhibits NO synthase, and reduced by oxyhaemoglobin (~70%). The apamin-insensitive ijps were partially restored (62.7±7.1% of control, n = 3, in response to a single pulse after 30 minutes perfusion) by L-arginine (0.1mM). Neurones in the myenteric plexus stained positively with NADPH diaphorase, known to label NO synthase (Dawson *et al* ,1991). The apamin-sensitive component was reduced by suramin (0.1mM) and reactive blue 2 (0.1mM) which have affinity for P_{2y} purinergic receptors while ATP (0.5μM) itself hyperpolarized and relaxed the sphincter. VIP (10nM) hyperpolarized and relaxed the sphincter but much more slowly than ATP. The VIP-induced hyperpolarization was unaffected by L-NAME (0.1mM), in the presence or absence of apamin, indicating that VIP may be acting independently of NO release.

Relaxation of the gpIAS is accompanied by a membrane hyperpolarization which comprises two components, both of which contribute to relaxation - an apamin - insensitive one which is likely to be mediated by NO and an apamin - sensitive component which may involve purines and/or peptide transmitters.

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M.G. Rae holds holds an M.R.C. / Pfizer studentship.

65P CHRONIC EXPOSURE TO THE NITROVASODILATORS GLYCERYL TRINITRATE (GTN) OR 3-MORPHOLINOSYDNONIMINE (SIN-1) INHIBITS PROSTACYCLIN RELEASE FROM ENDOTHELIAL CELLS

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Inhibition of the bradykinin-stimulated release of prostacyclin (PGI_2) from cultured endothelial cells has previously been demonstrated following both acute (Keen *et al.*, 1990) and chronic (Matthews *et al.*, 1993) exposure to sodium nitroprusside. In this study the effect of two further nitrovasodilators, GTN (Ignarro *et al.*, 1981) and SIN-1 (Feelisch & Noack, 1987), on basal and bradykinin-stimulated release of PGI_2 from the bovine aortic endothelial cell line AG4762 has been examined.

AG4762 cells (passage 16-23) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum and 1.5mM glutamine and plated out onto 15mm multiwells. Confluent cells were treated chronically (18h) with either GTN (500μM) or SIN-1 (500μM) in complete medium. Parallel studies were conducted in each case with appropriate vehicle. The cells were then incubated for 10min at 37°C in DMEM/Ham's Nutrient Mixture F-12 (1:1) containing either vehicle, 30nM or 100nM bradykinin. The release of PGI_2 was assessed by radioimmunoassay of the stable hydrolysis product 6-keto-$PGF_{1\alpha}$ (Crossman *et al.*, 1987). The statistical significance ($p \leq 0.05$, denoted by *) of the observed reduction in the levels of 6-keto-$PGF_{1\alpha}$ was assessed according to Student's t-test for paired data.

Table 1 PGI_2 release from AG4762 cells (mean ± s.e.mean, n=6)

Bradykinin (nM)	ng 6-keto-$PGF_{1\alpha}$/mg protein 0 (basal)	30	100
GTN vehicle control	8.9±1.9	42.7±14.3	38.5±12.2
GTN (500μM)	5.7±1.1*	21.5±6.3*	19.2±5.8*
SIN-1 vehicle control	9.1±2.5	41.4±14.0	44.5±16.3
SIN-1 (500μM)	2.6±0.5*	9.6±2.7*	11.0±2.8*

Pretreatment with either GTN or SIN-1 reduced basal levels of 6-keto-$PGF_{1\alpha}$ (31% and 66% respectively), and also reduced the increased levels in response to both 30nM bradykinin (44% and 75% respectively) and 100nM bradykinin (45% and 67% respectively). These results demonstrate an inhibitory effect on both basal and agonist-stimulated PGI_2 release from endothelial cells following chronic exposure to GTN or SIN-1.

Our thanks to Cassella AG for the generous gift of SIN-1 and to the Wellcome Trust for financial support.

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66P COMPARISON OF THE HAEMODYNAMIC PROFILES OF STRUCTURALLY-UNRELATED NITROVASODILATORS IN ANAESTHETISED DOGS

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The nitrovasodilators represent an important group of drugs in the treatment of cardiovascular disease. We have compared in the anaesthetised dog the systemic and coronary haemodynamic effects of various putative nitrovasodilators representing different chemical classes: nitroglycerin (NTG), sodium nitroprusside (SNP), linsidomine (SIN-1) and FK409, (±)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (Yamada *et al.*, 1991).

Chloralose-anaesthetised, artificially-ventilated dogs were set up to measure left circumflex coronary artery diameter (CD; sonomicrometry) and blood flow (electromagnetic), systolic aortic pressure (SAP), left ventricular end diastolic pressure (LVEDP) and dP/dt max, and heart rate (HR). Total peripheral and coronary vascular resistances (TPR, CVR) were calculated. NTG (n=15), SNP (n=5) and FK409 (n=5) (each 0.03 to 30 μg kg^{-1}) and SIN-1 (n=5) (0.01 to 1 mg kg^{-1}) were administered as i.v. bolus injection. Each dog received NTG (standard) and one of the other agents.

NTG, SNP and FK409 induced dose-dependent decreases in CVR (max effects: -58.8 ± 1.4%, -53.2 ± 6.3%, -52.6 ± 3.0%, respectively) and dose-dependent increases in CD (max effects: 14.6 ± 1.4%, 13.8 ± 1.4%, 15.7 ± 3.3%, respectively). This vasodilation of small and large coronary arteries was associated with dose-dependent decreases in SAP, LVEDP and TPR, and with increases in both HR and dP/dt max which were presumably reflex in origin. The dose-response curves for large coronary artery dilation to NTG, SNP and FK409 were superimposable. Considering the vasodilation of small coronary arteries (decrease in CVR), NTG and SNP were approximately 3 times more potent than FK409. SIN-1 dose-dependently increased CD (max effect: 16.3 ± 3.7 %) and was 30 times less potent than NTG in this respect, however SIN-1 had no effect on CVR. SAP, LVEDP and TPR decreased following SIN-1 administration but HR and dP/dt max were largely unchanged. At the level of the large coronary artery, NTG, SNP, FK409 and SIN-1 showed differences in the kinetics of their vasodilating effects. The times to peak effect on CD were 1, 1.5, 2 and 15 min, respectively, and the times for 50% recovery were 3, 5, 9 and 30 min, respectively. These results indicate that NTG, SNP and FK409 exhibit similar systemic and coronary haemodynamic effects with short durations of action, however SIN-1 selectively dilates large rather than small coronary arteries and this effect is relatively long lasting. Therefore, nitrovasodilators can demonstrate different haemodynamic profiles.

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67P CAPTOPRIL INCREASES MICROVASCULAR BLOOD FLOW IN ANAESTHETISED RABBIT SKIN BY A BRADYKININ-DEPENDENT MECHANISM

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Angiotensin converting enzyme inhibitors are useful in the treatment of heart failure. The site of action is not known, but may involve the microcirculation. We have determined that captopril increases local microvascular blood flow in rabbit skin *in vivo* and investigated the mechanism using a bradykinin antagonist (BK Ant) Nα-adamantaneacetyl-D-Arg[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin (Lammek *et al.*,1990), and inhibitors of cyclooxygenase and nitric oxide synthase.

Test agents were injected intradermally in the shaved dorsal skin of New Zealand White rabbits anaesthetized with sodium pentobarbitone 30 mg kg. Local skin blood flow was measured with a laser Doppler flow probe before and 30 min after injection of test agents (Warren *et al.*,1993). Experiments were repeated four times in each animal.

Captopril over the dose range 10^{-12} - 10^{-8} moles per site increased local basal blood flow from 35±13% to 92±14% (n=5 rabbits). In a further six rabbits, captopril 10^{-11} and 10^{-10} moles per site increased basal blood flow by 39±8% and 43±14% respectively and this was inhibited significantly ($P<0.05$) by co-injecting with either a) the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) 10^{-7} moles per site (-8±9 and -7±14% respectively); b) indomethacin $3x10^{-9}$ moles per site (-11±4% and -17±3% respectively); or c) BK Ant 10^{-10} moles per site (16±7% and 17±7% respectively). Injection of control buffer, L-NAME 10^{-7} moles per site, indomethacin $3x10^{-9}$ moles per site or BK Ant 10^{-10} moles per site alone changed basal blood flow by -2±6%, -9±9%, -14±5% or -2±8% respectively. Co-injection of BK Ant 10^{-10} moles per site abolished the blood flow response to bradykinin 10^{-11} moles per site. Data are mean ±s.e.mean. The intradermal injection of captopril up to doses of 10^{-5} moles per site did not affect systemic arterial blood pressure measured by carotid artery cannulation of an anaesthetized rabbit.

Captopril increases microvascular blood flow in rabbit skin *in vivo*. The present data are consistent with this being secondary to the inhibition of kininase II leading to an increase in endogenous tissue bradykinin concentrations, with subsequent stimulation of prostaglandin and nitric oxide release.

This work was supported by the British Heart Foundation.

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68P INTERACTIONS BETWEEN ENDOGENOUS NITRIC OXIDE, PLATELET ACTIVATING FACTOR AND THROMBOXANES DURING ENDOTOXIN-INDUCED ACUTE VASCULAR INJURY IN RAT INTESTINE

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The nitric oxide (NO) synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA) augments the acute intestinal vascular injury induced by high doses of endotoxin (Hutcheson et al, 1990). Furthermore, both L-NMMA and the more potent N^G-nitro-L-arginine methyl ester (L-NAME) provoke acute intestinal vascular injury when administered concurrently with low doses of endotoxin which themselves do not induce damage (Laszlo et al, 1993). The role of the pro-inflammatory and injurious mediators, platelet-activating factor (Paf) and thromboxanes (TX) in such intestinal damage, has now been evaluated.

Under halothane anaesthesia, *E. coli* lipopolysaccharide (LPS; 3 mg kg^{-1} i.v.) was administered to rats (male, 225-275g). [^{125}I] human serum albumin (2μCi kg^{-1} i.v.) was administered concurrently for the subsequent determination of vascular albumin leakage as an index of vascular injury, 1h after challenge. Neither LPS administration nor L-NAME (5 mg kg^{-1} s.c.) alone affected resting vascular permeability, but their concurrent administration substantially augmented albumin leakage in the ileum and colon (by Δ464±14 and 229±5 μl plasma g^{-1} tissue respectively, mean±s.e.mean, n=12, $P<0.001$ for both by ANOVA). Prior administration (15 min) of the Paf-receptor antagonists, WEB 2086 (0.1-1 mg kg^{-1} s.c.) or BN 52021 (2.5-10 mg kg^{-1} s.c.) dose-dependently reduced this vascular injury, being inhibited by 76±8 and 95±2% in the ileum, and by 83±2 and 91±3% in the colon, with the higher dose of each agent respectively ($P<0.001$ for each). Pretreatment with the TX-receptor antagonist BM 13177 (2 mg kg^{-1} s.c.) also reduced the vascular injury in the ileum and colon by 91±5 and 92±4% respectively ($P<0.001$). Likewise, pretreatment with the TX synthase inhibitors, 1-benzyl-imidazole (50 mg kg^{-1} s.c.) or OKY 1581 (10 mg kg^{-1} s.c.) abolished such vascular injury in the ileum and colon ($P<0.001$), suggesting synergistic interactions between the damaging actions of endogenous Paf and thromboxanes.

Previous studies using such Paf-receptor antagonists or TX synthase inhibitors have demonstrated a role for these mediators in the acute tissue necrosis and vascular injury induced in the gastrointestinal tract by high doses of endotoxin (Wallace et al, 1987; Boughton-Smith et al, 1989). The present study suggests that Paf and thromboxanes are also released during the initial phase following low dose endotoxin challenge, but do not injure the microvasculature unless released in the presence of a NO synthase inhibitor. Such findings thus further support the protective role of constitutively-formed NO in the modulation of microvascular integrity under acute pathological conditions.

F. Laszlo was supported by an E.C. fellowship

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69P NITRIC OXIDE-INDUCED NEUROGENIC VASODILATATION IN THE RAT SKIN

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Neurogenic vasodilatation induced by topical application of mustard oil (5 %) to the rat skin depends in part on the formation of nitric oxide (NO) (Lippe *et al.*, 1993). The present study examined in which way NO participates in the vasodilator response to mustard oil. The experiments were performed on rats anaesthetized with urethane. Blood flow in the plantar skin of the hindpaws was assessed by laser Doppler flowmetry. The participation of NO in the processes under study was tested by the use of the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 15 mg kg^{-1}) which was given i.v. because intraplantar administration of L-NAME caused a marked reduction of cutaneous blood flow, which was not seen after i.v. injection.

Topical administration of mustard oil (5 % in paraffin oil) to saline-treated rats increased blood flow by 432 ± 69 % (mean ± s.e. mean, n = 6) whereas in rats treated with L-NAME mustard oil raised blood flow by 193 ± 63 % (n = 6) only. This inhibitory effect of L-NAME ($P < 0.05$) was not shared by the inactive enantiomer D-NAME. L-NAME, however, failed to inhibit the cutaneous vasodilatation evoked by antidromic stimulation (pulses of 1 ms, 15 V, delivered at 2 Hz for 30 s) of the cut saphenous nerve, which in the absence of L-NAME increased blood flow by 137 ± 31 % (n = 10). L-NAME also failed to inhibit the cutaneous vasodilator response to i.v. injection of calcitonin gene-related peptide (CGRP, 0.1 - 1 nmol kg^{-1}, n = 6), which is supposed to be the major mediator of afferent nerve-mediated vasodilatation in the skin (Brain *et al.*, 1993).

The inhibitory effect of L-NAME was absent in rats pretreated with a neurotoxic dose of capsaicin (125 mg kg^{-1} s.c.) 2 weeks before the experiments (n = 9), which indicates that L-NAME inhibited the neurogenic component of the vasodilator response to mustard oil. Pretreatment of rats with capsaicin reduced the mustard oil-induced hyperaemia by about 43 % (n = 11). Intraplantar administration of the NO donor sodium nitroprusside (SNP, 0.15 nmol in 15 µl) enhanced blood flow by 135 ± 20 % (n = 20). The hyperaemic reaction to SNP was attenuated by 42 % (n = 14, $P < 0.05$) in rats pretreated with capsaicin. Likewise, the CGRP receptor antagonist $CGRP_{8-37}$ (50 nmol kg^{-1} i.v.) was also able to reduce the SNP-evoked vasodilatation by 41 % (n = 6, $P < 0.05$).

These results indicate that NO participates in the neurogenic hyperaemic reaction of the skin to chemical irritants such as mustard oil. The role of NO, however, is not to be a vasodilator mediator but to contribute to the mustard oil-induced excitation of afferent nerve endings. Such a role of NO is substantiated by the observations with the NO donor SNP whose vasodilator action depends also in part on stimulation of CGRP-releasing afferent nerve fibres.

Supported by the Austrian Research Foundation (grant no. P9473-MED).

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70P DIFFERENTIAL EFFECT OF NITRIC OXIDE DONORS ON THE DISTRIBUTION OF THE COMMON CAROTID BLOOD FLOW IN THE CRANIAL CIRCULATION OF THE PIG

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Cranial arteriovenous anastomotic (AVA) dilatation has been implicated in the pathogenesis of migraine, though the mechanism involved is not fully understood. Recently, nitric oxide (NO) has been proposed to have a role in migraine. Thus, inhibition of NO-synthesis reduces AVA blood flow in pigs (Van Gelderen & Saxena, 1993) and nitrovasodilators, known to release NO, cause temporal artery dilations and headache in humans, being most pronounced in migraineurs (Olesen *et al.*, 1993). The purpose of the present study was therefore to evaluate the effect of intracarotid infusions of the NO donors, sodium nitroprusside (NPR) and S-nitroso-N-acetylpenicillamine (SNAP), on the distribution of the common carotid blood flow in anaesthetized pigs with low AVA blood flow (Den Boer *et al.*, 1993). The distribution was measured with repeated injections with radioactive microspheres in the common carotid artery. Three groups of pigs, instrumented to measure haemodynamic variables, including carotid blood flow and its distribution, were treated with either saline (4 times; n=5), NPR (3, 10, 30, 100 µg min^{-1}; n=4) or SNAP (3, 10, 30, 100 µg min^{-1}; n=6).

Table 1. Carotid blood flow distribution (ml min^{-1}) after NPR or SNAP (µg min^{-1}).

	Baseline	3	10	30	100
Total carotid					
NPR	66±12	78±12	88±10*	102±10*	115±16*
SNAP	52±6	65±9	77±9*	91±4*	107±13†
AVA					
NPR	16±9	13±6	12±5	11±5	11±6
SNAP	14±4	21±8	26±9†	28±8†	n.d.
Total capillary					
NPR	50±5	65±9*	75±10*	91±8*	103±12*
SNAP	37±3	44±7	51±6†	62±6*	n.d.

n.d., not determined. †, $p<0.05$ vs. baseline; *, $p<0.05$ vs saline.

Infusion of saline caused no changes in systemic or carotid haemodynamics. NPR and SNAP increased heart rate (55±5% and 44±17%, respectively) with the highest dose only. Both drugs increased total carotid (maximum 64±16% and 85±18%, respectively) and total capillary (maximum 91±8% and 71±19%, respectively) blood flows (Table 1). In contrast to NPR, SNAP increased AVA blood flow (245±151%). In case of NPR both extracerebral (76±23%) and total brain (82±19%) blood flow increased, whereas SNAP enhanced extracerebral (84±17%) blood flow only. The latter increased dural blood flow dose-dependently (maximum 155±57%). Both drugs enhanced vascular pulsations (74±25% and 109±45%, respectively). It is concluded that both of these NO donors cause arteriolar dilatation in the cranial circulation of the pig, but only SNAP dilates AVAs.

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71P INHIBITION OF EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE AND REDUCTION OF BLOOD FLOW IN MURINE TUMOURS BY DEXAMETHASONE

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Angiogenesis is an integral part of tumour growth but tumour vessels differ morphologically and pharmacologically from control neovasculature. They are often contorted, saccular, widely dilated and unresponsive to vasodilator agonists. We have previously shown that nitric oxide (NO) produced by an inducible NO synthase (iNOS) regulates blood flow in a murine adenocarcinoma colo 26 hosted in sponge implants in Balb/c mice. L-arginine analogues which inhibit NOS significantly reduce tumour blood flow (Andrade & Piper 1992) and iNOS immunoreactivity is present in tumour vasculature (Buttery et al. 1993); we have now investigated whether dexamethasone, which inhibits expression of iNOS, alters tumour blood flow in this sponge model in vivo.
Balb/c mice were anaesthetised with 0.315 mg ml^{-1} fentanyl citrate and 10 mg ml^{-1} fluanisone acetate and 5 mg ml^{-1} midazolan hydrochloride and polyether polyurethane sponge discs (8mm x 4mm) were implanted subcutaneously . Three days postimplantation 1 x 10^6 colo 26 tumour cells were injected into the sponges (day 0). Local blood flow in the implants was assessed by washout of ^{133}Xe. Groups of tumour-bearing mice (n=6) were given dexamethasone (0.1-1.0 mg kg^{-1} IM) daily from day 3-14 when the experiment was terminated. Control tumour-bearing mice (n=6) received saline daily IM. T½ was measured on days 7,10 and 14. Animals were killed on day 14 and implants were removed, weighed, fixed in 1% paraformaldehyde and frozen sections immunostained with rabbit antiserum to iNOS (Hamid et al. 1993).
Following treatment with dexamethasone 1.0 mg kg^{-1} for 4 days, T½=18.78±3.11 and this was significantly greater than for controls T½=5.32±2.16 ($p<0.05$). After 7 and 11 days treatment dexamethasone 1 mg kg^{-1} increased T½ (T½=7.16±0.2) compared with control T½=3.38±0.51 ($p<0.001$). Total weight of tumour and sponge was decreased by dexamethasone 0.5 (0.77g±0.25g) and 1mg kg^{-1} (0.53g±0.05g) compared with controls (1.86g±0.25g) ($p<0.02$ and, 0.01 respectively). Strong immunostaining was seen in the vasculature of untreated tumour-bearing implants, but only moderate and negligible staining was seen after treatment with 0.5 and 1.0 mg kg^{-1} dexamethasone, respectively.
These results show that expression of iNOS in colo 26 tumours in mice is dose dependently inhibited by dexamethasone and this results in reduced blood flow in tumour vasculature.

We thank the Cancer Research Campaign for financial support and Dr I.R. Hart for the colo 26 cells
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72P OXIDISED, BUT NOT NATIVE, LOW DENSITY LIPOPROTEIN ACTIVATES ENDOTHELIAL CELL PROTEIN KINASE C

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We have previously shown that the specific protein kinase C (PKC) inhibitor Ro 31-8220 (3- [1- {3-amidinothio} propyl] -3- indolyl) -4- (1-methyl-3-indolyl)-1H-pyrrole- 2,5-dione methanesulphonate) prevents the acute inhibition of EDRF release by oxidised LDL (oxLDL) in isolated rings of rabbit aorta (Smith & Turner, 1992), and suggested that oxLDL, like phorbol esters, acts by stimulating PKC. We now provide direct evidence that oxidised, but not native, LDL stimulates PKC in porcine cultured aortic endothelial cells. PKC was assayed radioenzymatically, utilising transfer of ^{32}P-labelled phosphate from ATP to a protein substrate (Lang & Lewis, 1992). Exposure of cells to native LDL (0.1 mg protein ml^{-1}) did not significantly alter cytosolic or particulate PKC activity over the 10 min exposure period (Table 1). Oxidised LDL (0.1 mg protein ml^{-1}) however significantly increased both cytosolic and particulate PKC activity for up to 9min (Table 1). In a separate series of experiments, the oxLDL-induced (0.1 mg protein ml^{-1} for 1min) increase in both cytosolic and particulate PKC activity (from 0.38±0.15 and 0.18±0.04 to 0.81±0.13 and 1.12±0.24 respectively, $p<0.05$) was prevented by pretreating the cells with Ro 31-8220 ($10^{-5}M$, 10min), cytosolic=0.26±0.06, particulate=0.22±0.24. The evidence supports the suggestion (Smith & Turner, 1992) that oxLDL inhibits agonist-induced EDRF release by stimulating PKC.

Table 1. Time course of effects of native or oxidised LDL on cultured endothelial cell PKC activity. Results (pmoles of ^{32}P-phosphate transferred to substrate min^{-1}) are expressed as mean±s.e. mean. *=$p<0.05$ and #=$p<0.01$ cf time zero.

	Time (min)	0	0.5	1	2	5	10
LDL type	PKC fraction						
Native	Cytosolic	1.27±0.28	0.7±0.14	0.86±0.19	1.35±0.45	1.19±0.4	0.77±0.23
Native	Particulate	1.21±0.3	0.66±0.15	0.95±0.26	1.44±0.34	0.98±0.22	0.71±0.22
Oxidised	Cytosolic	0.12±0.013	0.25±0.025	0.31±0.043*	0.44±0.089#	0.36±0.033#	0.35±0.047#
Oxidised	Particulate	0.11±0.02	0.32±0.094	0.38±0.07*	0.47±0.091#	0.33±0.05	0.29±0.049

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73P THE EFFECT OF STREPTOZOTOCIN-INDUCED DIABETES ON AGONIST-STIMULATED AND FLOW-STIMULATED RELEASE OF NITRIC OXIDE IN THE MESENTERIC CIRCULATION OF THE RAT

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The pathophysiology of the vascular complications of diabetes mellitus has been the subject of much study. Aortic rings from rats with streptozotocin (STZ)-induced diabetes relax less well to agonists which stimulate nitric oxide (NO) release from the endothelium (Oyama et al., 1986). Shear-stress also influences the release of NO, an important factor in diabetes as blood viscosity increases in this disease. This study examined the effect of STZ-induced diabetes on agonist-induced and flow-induced release of NO in the mesenteric circulation of the rat.

Male wistar rats (250-350 g; n=5 or 6 per group) were injected with 70 mg Kg^{-1} STZ (i.p.). Age-matched controls received saline. After 7 to 14 days, rats were anaesthetised and one of two experiments performed. Either the mesenteric arterial bed was isolated and perfused with oxygenated modified Krebs Henseleit solution according to the method of McGregor (1965) or the rats were prepared as described by Randall & Hiley (1988) to study pressure /flow relationships in the *in situ* blood perfused mesenteric preparation. In the first case, dose response curves to carbachol (Cch) were obtained by co-administering Cch (0.02 to 4 n moles) with a standard submaximal dose of noradrenaline (NA) which, when administered alone, gave an increase in perfusion pressure of 80 to 100 mm Hg. In the second case, the perfusion pressure was recorded at flow rates of 0.33, 0.96, 1.34, 1.96 and 2.6 ml min^{-1}. The animals then received N^G-nitro-L-arginine methyl ester (L-NAME; 10 mg Kg^{-1} i.v.) and the perfusion pressure at each flow rate was recorded.

All rats injected with STZ had urinary glucose concentrations of >110 mM whereas control rats had no glucosuria. The isolated Krebs perfused mesenteries from diabetic rats were less sensitive to Cch when compared with control preparations with respective EC_{50} values of 0.13 ± 0.01 and 0.09 ± 0.01 nmole and maximum responses of 72.8 ± 0.66 vs 85.8 ± 2.1 percent reduction in NA-induced rise in pressure ($p < 0.05$ for both parameters; unpaired *t*-test). In the *in situ* blood perfused mesentry, diabetic preparations had a significantly lower perfusion pressure (mm Hg) when compared with controls at flow rates of 1.34 (30.3 ± 0.9 vs 37.8 ± 2.3; p=0.01), 1.96 (35.3 ± 1.5 vs 44.4 ± 2.3; $p<0.01$) and 2.6 ml min^{-1} (39.0 ± 1.9 vs 49.2 ± 2.2; $p<0.01$ using an unpaired *t*-test). After L-NAME administration, the pressure at all flow rates increased significantly compared with pre-treatment values ($p<0.05$; paired *t*-test) in both control and diabetic preparations. The rise in perfusion pressure (mm Hg) obtained with L-NAME did not differ significantly between diabetic and control preparations at any flow rate tested (5 ± 1.8 vs 7.8 ± 1.9, 5.5 ± 2 vs 6.8 ± 2.1, 9.1 ± 1.7 vs 8.2 ± 2.1, 16.8 ± 5.5 vs 10.7 ± 3.6 and 22 ± 7.5 vs 13.9 ± 3.8 at flow rates of 0.33, 0.96, 1.34, 1.96 and 2.6 ml min^{-1}; control vs diabetic respectively)

The apparent deficiency in NO production by endothelial cells following agonist stimulation (seen here and reported by others) may occur at the receptor or second messenger level since flow-induced release of NO is not reduced in the same vascular bed. Indeed, these data suggest flow-induced release of vasodilators increases in diabetes.

This work was funded by a grant from the Nuffield Foundation.

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74P IMPAIRMENT OF ARTERIOLAR SENSITIVITY TO ILOPROST IN DIABETIC RATS: PARTIAL RESTORATION BY CHRONIC TREATMENT WITH TRANDOLAPRIL

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Diabetes mellitus is known to be associated with microvascular lesions and impaired responsiveness to various hormones and drugs, particularly to prostaglandins (Hill and Larkins, 1987). It is also associated with an augmentation of angiotensin converting enzyme (ACE) activity, which may in turn increase basal tone and arteriolar wall thickness (Porta et al., 1987). The link between prostaglandin responsiveness and ACE activity is not yet well established. The aim of this study was to test the effects of trandolapril, an ACE inhibitor, on arteriolar reactivity to prostacyclin. Four groups of rats weighing 214 ± 2 g , were studied. One group was kept as a control. The other 3 groups received a single i.v. injection of 40 mg/kg of streptozotocin, to induce diabetes, and rats having a glycemia between 2 and 5 g/l were included in the study. Two weeks after diabetes induction, among the diabetic groups, one received water p.o., and the others respectively 30 and 300 µg/kg/day of trandolapril p.o. for four weeks. Rats were anesthetized with pentobarbital sodium and the cremaster muscle microvessels were observed using intravital microscopy, as described elsewhere (Vicaut and Stücker, 1990). No difference in arterial blood pressure was found between groups. The preparation was sequentially superfused with iloprost, a synthetic prostacyclin, at 10 nM and 0.1 µM, mefenamic acid 20 µM a cyclooxygenase inhibitor, and both papaverine and sodium nitroprussiate at 0.1 mM each to achieve maximal vasodilation and therefore assess dilation reserve (DR). Percentages of treated to basal variation in diameters were calculated on 8 to 27 arterioles per group, included in the 8 to 20 µm diameter range, and are reported hereunder:

	Basal tone DR	Iloprost 10 nM	Iloprost 0.1 µM	Mefenamic acid
Control	21± 3	13 ± 4	25 ± 4	- 13 ± 7
Diabetic	40 ± 5	5 ± 3	2 ± 4	4 ± 3
Diabetic trandolapril 30	32 ± 6	10 ± 5	19 ± 5	- 15 ± 5
Diabetic trandolapril 300	30 ± 4	20 ± 5	20 ± 5	- 1 ± 6

As compared to control diabetic rats exhibit a significant increase ($p \leq 0.02$) in arteriolar basal tone. This result suggests that diabetes induced a basal vasoconstriction. Diabetic trandolapril treated groups were not significantly different either from control or from diabetic groups. Despite the increase in basal tone, diabetic groups did not respond to iloprost stimulation. Following iloprost 0.1 µM superfusion, there were significant differences respectively between diabetic and control groups ($p \leq 0.03$), and between diabetic treated and diabetic untreated groups ($p \leq 0.05$). Inhibition of cyclooxygenase by mefenamic acid induced significantly different opposite effects between diabetic and control groups ($p \leq 0.01$): a constriction was observed in the control group and a slight dilation in the diabetic one. Treatment with trandolapril did not significantly change the response. We conclude that diabetes impairs the production of prostaglandins and the sensitivity to prostacyclin at the microcirculatory level. Trandolapril seems able to partially reestablish the reactivity of precapillary arterioles to prostacyclin stimulation without modifying the glycemia in diabetic treated groups as compared to the diabetic untreated one.

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75P BIOCHEMICAL PHARMACOLOGY IN THE RAT OF THE NOVEL ALDOSE REDUCTASE INHIBITOR ZD5522

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Aldose reductase (ALR2; EC 1.1.1.21), a key enzyme of the polyol pathway, has been implicated in the pathogenesis of the long term complications of diabetes mellitus (for review see Tomlinson,1993). In the diabetic state, ALR2 converts glucose to sorbitol which, in turn, is converted to fructose by sorbitol dehydrogenase. To date results of clinical trials of ALR2 inhibitors have been disappointing and do not confirm the proven functional benefit seen in experimental models of diabetic complications eg. improvement in electrophysiological deficits in motor and sensory nerve conduction velocities. Consequently, attention has focussed on the requirement for a very high and sustained level of ALR2 inhibition in affected tissues in order to block flux through the polyol pathway and provide a definitive test of the 'ALR2 hypothesis' (Cameron & Cotter, 1992).

ZD5522 (3',5'-dimethyl-4'-nitromethylsulphonyl-2-(2-tolyl)acetanilide) is a representative of a new class of ALR2 inhibitors containing the sulphonylnitromethane pharmacophore. Under standard conditions: 67mM phosphate/NaOH, pH 6.2; 75μM NADPH; 0.4 $LiSO_4$, 5μl CH_3SOCH_3/ml. The compound is a pure non-competitive inhibitor of bovine lens ALR2, having a Ki of 7.2 ± 0.6 nM. In an isolated rat sciatic nerve preparation, using galactose as substrate, ZD5522 caused a dose-related inhibition of dulcitol accumulation and had an IC_{95} of 11 uM. When dosed p.o., once daily for 5 consecutive days to male streptozotocin (STZ) diabetic rats, ZD5522 caused a dose related inhibition of sciatic nerve sorbitol and fructose levels. Briefly, levels of sugars in sciatic nerve were measured by gas chromatography of silyated derivatives following extraction (by boiling) and precipitation ofprotein. The ED_{95} for sorbitol inhibiton was 2.8 mg/kg/day; measured 24 hr after the last dose. At a dose of 5.6 mg/kg/day the values for sciatic nerve sorbitol and fructose levels (27.6 ± 2.6 and 237.8 ± 6.8 ug/g wet wt tissue, mean ± s.e.mean; n=6, respectively) were reduced from (490.7 ± 35.1 and 1318.0 ± 32.5 ug/g wet wt tissue, mean ± s.e.mean; n=12, respectively) in control, non-treated STZ diabetic rats.

Thus, ZD5522 is an effective, orally active ALR2 inhibitor which causes a sustained and high level of ALR2 inhibiton in rat sciatic nerve when administered once daily.

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76P PAF-INDUCED EOSINOPHILIA, BUT NOT LPS-INDUCED NEUTROPHILIA, IN GUINEA-PIG LUNG IS MEDIATED BY IL-5

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Pretreatment of sensitised guinea-pigs with a monoclonal antibody to human interleukin-5, (IL-5), TRFK5, has been shown to inhibit antigen-induced eosinophil accumulation in guinea-pig lung, suggesting that IL-5 mediates this response (Chand *et al*, 1992; Gulbenkian *et al*, 1992; Sanjar *et al*, 1992). Exposure of naive guinea-pigs to aerosols of platelet activating factor (PAF) also causes bronchial eosinophilia (Whelan & Johnson,1992). In the present study, we have used TRFK5 to investigate whether or not IL-5 also mediates PAF-induced eosinophilia. The effects of TRFK5 on lipopolysaccharide (LPS)-induced neutrophilia were also investigated.

PAF-induced eosinophilia and LPS-induced neutrophilia in guinea-pig lung were determined as described previously (Whelan & Johnson,1992). All data for cell counts are presented as means ± s.e.mean of the number of observations shown in parentheses.

Twenty four hours after inhalation of PAF (100μg.ml^{-1}), the bronchoalveolar lavage fluid (BALF) eosinophil count increased from 24.7 ± 3.6 to 173.0 ±11.3 cells.μl^{-1} (n=6). TRFK5 (10 to 100μg.kg^{-1} i.p.), 1h prior to challenge, caused a dose-related inhibition of PAF-induced eosinophilia, with the highest dose of TRFK5 used (100μg.kg^{-1}) significantly ($P<0.05$; Student t test) reducing the BALF eosinophil count to 86.2 ±13.7 cells.μl^{-1} (n=6). Four hours after inhalation of LPS (100μg.ml^{-1}), the BALF neutrophil count increased from 2.5 ± 2.5 to 354.5 ± 27.6 cells.μl^{-1} (n= 6). TRFK5 (100μg.kg^{-1} i.p.), 1h prior to LPS, had no significant effect ($P>0.05$) on LPS-induced neutrophilia (neutrophil count: 351.2 ± 36.1 cells.μl^{-1}, n=6). In unchallenged animals, TRFK5 (100μg.kg^{-1}) had no significant effect ($P>0.05$) on the leukocyte count of the BALF.

The data show that TRFK5 reduces PAF-induced eosinophilia over the same dose-range as that used by others to inhibit antigen-induced eosinophilia, indicating that IL-5 is an important mediator of PAF-induced eosinophil accumulation in the guinea-pig lung. In contrast, IL-5 does not appear to mediate LPS-induced airway neutrophilia in this species.

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77P A NOVEL EOSINOPHIL-SPECIFIC CHEMOATTRACTANT CYTOKINE GENERATED IN AN *IN VIVO* MODEL OF ALLERGIC ASTHMA IN THE GUINEA-PIG

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Eosinophil accumulation in the lung is a prominant feature of allergic asthma which has been correlated with airway dysfunction. Although much is known about the spasmogens involved in the acute response to allergen provocation, the mediators involved in the subsequent induction of eosinophil accumulation are unknown.

We have addressed this issue using a strategy previously developed to detect neutrophil chemoattractants in an *in vivo* animal model (Beaubien *et al.*, 1990; Collins *et al.*, 1991; Jose *et al.*, 1991). Sensitised guinea-pigs were challenged with aerosolised antigen to generate activity in the lung *in vivo* and guinea-pig skin was used as an *in vivo* bioassay. Guinea-pigs were sensitised by intraperitoneal ovalbumin followed by an aerosol boost at day 8. The animals were challenged with aerosolised antigen at day 15-21 and bronchoalveolar lavage (BAL) fluid was collected at intervals up to 24h. BAL fluid was injected intradermally in unsensitised assay guinea-pigs previously injected intravenously with ^{111}In-eosinophils (Faccioli *et al.*, 1991). Counts in excised skin samples were measured after a 30min, 120min or 240min accumulation period. Activity was detectable in 30min BAL samples, rising to a peak at 3-6h and falling to low levels by 24h. The activity was purified by a series of HPLC steps, using the skin system as a bioassay throughout. Final purification resulted in two peaks of chemoattractant activity. The N-terminal 29 amino acid sequences were identical for the two peaks and laser desorption time of flight mass analysis suggested a difference in glycosylation. The 73 amino acid primary structure was determined by additionally sequencing overlapping peptides from proteolytic digests. The peptide, 'eotaxin', is a novel C-C chemokine of the platelet factor 4 family, but without high homology with other known members. Eotaxin was highly potent in inducing ^{111}In-eosinophil accumulation; a 1.6pmol dose inducing 1806±476 cells per site over a 30min or 2943±302 over 240min accumulation period, compared with saline controls of 394±96 and 508±118, respectively (mean ± sem for n=4 guinea-pigs). Eotaxin did not induce ^{125}I-albumin leakage or ^{111}In-neutrophil accumulation. Significantly, eotaxin was potent in activating both guinea-pig and human eosinophils in assays of changes in intracellular Ca^{++} and chemotaxis *in vitro*.

We suggest that molecules related to eotaxin may have an important role in allergic diseases, such as asthma, in man.

The National Asthma Campaign generously supported this project.

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78P INDUCTION OF CYCLOOXYGENASE-2 IN HUMAN PULMONARY EPITHELIAL CELLS

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Cyclooxygenase (COX) metabolises arachidonic acid to prostaglandins and exists in at least two different isoforms. COX-1 is present under physiological conditions, whereas COX-2 is induced by endotoxin or cytokines *in vitro* (Maier et al., 1990) and under inflammatory conditions *in vivo* (Vane et al., 1994). Here we have investigated the expression of COX-1 or COX-2 isoforms in human pulmonary epithelial cells after exposure to proinflammatory cytokines. The release of COX-metabolites (prostaglandin (PG) E_2, 6-keto $PGF_{1\alpha}$, $PGF_{2\alpha}$ and thromboxane B_2) was measured by radioimmunoassay (Salmon, 1978). Human primary pulmonary epithelial cells (HPE) and the human pulmonary epithelial cell line, A549, were obtained and cultured as previously described (Kwon et al., 1993). The isoform of COX present was measured in cell extracts by western blot analysis using selective antibodies to COX-1 or specific antibodies to COX-2 (Mitchell et al., 1993).

Untreated HPE or A549 cells contained no COX-1 or COX-2 protein and released low levels of COX-metabolites (<0.2 ng/ml over a 24 h incubation period). Endotoxin (1 μg/ml for 24 h) caused a small induction of COX-2 protein in HPE which was enhanced in the presence of interferon-γ, with no further increase in the presence of a cytokine mixture (CM; interleukin-1β, tumour necrosis factor-α and interferon-γ, 10 ng/ml for all). In contrast, A549 cells contained only low levels of COX-2 protein after exposure to endotoxin or endotoxin plus interferon-γ but contained large amounts of COX-2 protein after exposure to CM. In the presence of CM, A549 cells released greater amounts of COX metabolites (PGE_2, 8.80±0.72 ng/ml; 6-keto $PGF_{1\alpha}$, 0.62±0.07 ng/ml; thromboxane B_2, <0.2 ng/ml; $PGF_{2\alpha}$, 2.42±0.2 ng/ml; n=6-9). In the presence of CM HPE released only PGE_2 (0.3 ng/ml; n=6). The release of COX metabolites from A549 cells in response to CM was inhibited by pretreatment (30 min) with dexamethasone (1 μM; PGE_2, 0.52±0.03; 6-keto $PGF_{1\alpha}$, <0.2 ng/ml; $PGF_{2\alpha}$, <0.2 ng/ml; n=6). Dexamethasone also inhibited the induction of COX-2 protein in A549 cells treated with CM. Interleukin-1β was the main component of CM responsible for the increase in PGE_2 synthesis (interleukin-1β 10.84±0.43 g/ml; tumour necrosis factor-α, 0.25±0.02 ng/ml interferon-γ, 0.22 ±0.02; n=3).

These findings demonstrate that HPE and A549 contain undetectable levels of COX-1 under control conditions and release low levels of COX-metabolites. However, COX-2 is induced in the presence of cytokines with a accompanying release of metabolites which is sensitive to inhibition by dexamethasone. The role of COX-2 in human pulmonary disease is so far unclear. These findings may help in the understanding of the mechanism of action of steroids in pulmonary inflammation and lead to more specific therapies.

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79P NON-ADRENERGIC NON-CHOLINERGIC NEURAL RELAXATION IN HUMAN TRACHEAL SMOOTH MUSCLE IS MEDIATED BY AN INCREASE IN cGMP: FURTHER EVIDENCE FOR THE ROLE OF NITRIC OXIDE

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Human tracheal smooth muscle (HTSM) possesses a prominent non-adrenergic non-cholinergic (i-NANC) neural bronchodilatior response mediated by nitric oxide (NO; Belvisi *et al.*, 1992). NO released from the endothelium of blood vessels causes relaxation by activating guanylyl cyclase to elevate cGMP levels (Katsuki *et al.*,1977). We have investigated, *in vitro*, the cAMP/cGMP content of HTSM during i-NANC responses evoked by EFS and compared these with changes seen with the NO-donor SIN-1.

Strips of HTSM from 15 normal donors (age 11-45yrs, 8 male) for heart transplantation, with or without epithelium (EP), were placed in organ baths for measurement of isometric tension. Atropine (1μM), propranolol and indomethacin (both 10μM) were present throughout. EFS (10Hz, 0.5ms, 40V for 30s) was applied to evoke i-NANC responses and tissues were snap frozen in liquid nitrogen at various time points (0-600s). The NO synthase inhibitor L-N^G-nitroarginine methyl ester (L-NAME) ± L-/D-arginine, D-NAME, the peptidase α-chymotrypsin (α-CT) and tetrodotoxin (TTX) were added 30min before a test EFS. The effect of SIN-1 was studied and tissues were frozen at various time points (0-32min). In all cases tension was measured and tissues were frozen for assessment of cAMP/cGMP content. Nucleotides were extracted by homogenization in 0.5M trichloroacetic acid, neutralized, acetylated, diluted 1 in 5 and measured by RIA (Brooker *et al.*, 1979). Levels are described as fmol/mg of tissue.

EFS produced a time-dependent relaxation and concurrent rise in cGMP levels without affecting cAMP levels. Rises in cGMP were significant at 10, 30 and 60s after the start of EFS and was maximal at 30s (t=0s: relaxation=0mg, cGMP=9.9±0.7, cAMP=239±70; t=30s: relaxation=1068±64mg, cGMP=22.9±1.7, P<0.001, cAMP=211±15, NS-both n=11), which was chosen as the time point for further investigation. Neither basal or stimulated levels of cGMP were affected by EP removal. L-NAME (100μM) caused an inhibition of both relaxation (4.7±2.9% of control, P<0.001, n=5) and cGMP accumulation (8.0±0.9, P<0.001, n=5), which was partially attenuated by coincubation with 1mM L-arginine (relaxation=86.7±5.6% of control, NS; cGMP=17.6±2.7, NS-n=5) but not 1mM D-arginine (relaxation=9.3±6.8% of control, P<0.001; cGMP=9.2±1.1, P<0.001- n=4). D-NAME (100μM) and α-CT (2u/ml) had no effect on relaxation or cGMP levels, however TTX (3μM) inhibited both relaxation (5.5±3.9% of control, P<0.001, n=4) and cGMP increases (9.9±1.1, P<0.001, n=4). SIN-1 (1mM) also produced a time-dependent relaxation and increase in cGMP levels with both maximal at 8min (relaxation: 1331±399mg, n=4; cGMP: 1314.5±450.2, P<0.001, n=4), without affecting cAMP levels (240.7±86.9, NS, n=4).

These data suggest that i-NANC relaxation is mediated by a concurrent rise in cGMP, but not cAMP levels which occurs in the HTSM. This evidence, the stereoselective inhibition of these changes by L- but not D-NAME, the lack of effect of α-CT and the similar increases in cGMP seen with the NO-donor SIN-1 suggest that the increase of cGMP in HTSM is mediated by NO, with no evidence that vasoactive intestinal polypeptide is involved as there is no change in cAMP levels.

This work was supported by the National Asthma Campaign, Wellcome Trust, and the British Lung Foundation.

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80P INHIBITION OF CONTRACTION BY PKC INHIBITORS, Ro31-8220 AND Ro31-75490, IN SMALL AND LARGE BRONCHIOLES OF THE RAT

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The effects of the protein kinase C inhibitors Ro31-8220 and Ro31-7549 have not so far been studied on constriction of small bronchioles. We have investigated their effects on carbachol, 5-hydroxytryptamine (5HT) and 4ß-phorbol dibutyrate (4ß-PDBu) induced contractions. The studies involved small bronchioles of internal diameter 514μM ± 16, n=75 and large bronchioles of internal diameter 1417μM ± 38, n=61. The work was carried out using a Mulvaney-Halpen small vessel myograph.

Male Wistar rats (200-300g) were anaesthetised with ether and the heart and lungs removed. Bronchioles were dissected free of connective tissue and mounted on the myograph. The tissues were bathed in a modified Ringer's solution and gassed with 5% CO_2 in O_2 and stretched to give a maximum response to 75mM [K^+]. Results are expressed as a percentage of this response unless otherwise stated (T_K).

As previously reported (Chopra et al., 1993) 4ß-PDBu (1nM - 1μM) had no effect on tension. However after precontracting with 30mM [K^+], 500nM 4ß-PDBu caused a contractile response of 110.4 ± 7.0 T_K in the small bronchioles and 69.3 ± 6.5 T_K in the large bronchioles. Ro31-8220 caused a concentration dependent inhibition of the 30mM [K^+] + 500nM 4ß-PDBu response in both size preparations. 10μM Ro31-8220 completely inhibited the phorbol ester response (n=8). The effect of Ro31-7549 was also examined in small bronchioles and had a similar effect to Ro31-8220. Ro31-8220 also caused a concentration dependent inhibition of the contractile response to 500nM carbachol. 10μM Ro31-8220 decreased this response by 58.5 ± 9.4 % in small bronchioles (n=9) and by 12.2 ± 5.4% in large bronchioles (n=6). In small bronchioles 10μM Ro31-7549 decreased the 500nM carbachol response by 41.3 ± 7.5%.

Concentration response curves to carbachol (10nM - 50μM) and 5HT (10nM - 50μM) were carried out in the absence and presence of Ro31-8220. In small bronchioles 10μM carbachol gave a maximal contraction of 161.8 ± 3.0 T_K and an EC_{50} of 533.1 ± 68.9nM (n=9). Large bronchioles had a significantly smaller response of 140.5 ± 4.6 T_K $p<0.01$ and an EC_{50} of 686.3 ± 164.0nM (n=8). In small bronchioles 1μM Ro31-8220 altered the maximal carbachol response to 110.8 ± 3.9 T_K ($p<0.001$), EC_{50} 655.0 ± 90.4nM ($p>0.1$). 10μM Ro31-8220 caused a shift to the right in the carbachol concentration response curve (EC_{50} 986.1 ± 50.2nM, $p<0.001$) and altered the maximum response to 102.0 ± 4.5 T_K, $p<0.001$, (n=8). In large bronchioles 1μM Ro31-8220 had no significant effect on the concentration response curve ($p>0.1$) but 10μM Ro31-8220 reduced the 10μM carbachol response to 106.1 ± 5.2 T_K ($p<0.001$), EC_{50} 905.0 ± 81.2nM ($p>0.1$) (n=8).

The maximum contraction to 10μM 5HT was similar in both small and large bronchioles (small: 89.4 ± 6.7 T_K, n=6; large: 69.3 ± 5.2 T_K, n=6), whereas the EC_{50} was greater in large bronchioles (small: 793.3 ± 163.0nM; large: 1.2 ± 0.05μM; $p<0.01$). In small bronchioles Ro31-8220 (10μM) caused both a shift to the right in the concentration response curve (EC_{50} 1.3 ± 0.09μM, $p<0.05$), and reduced the maximum response to 52.2 ± 4.7 T_K, ($p<0.01$). In large bronchioles 10μM Ro31-8220 had no significant effect on either the maximal 5HT response or the EC_{50}, (52.3 ± 6.0 T_K, 1.1 ± 0.06μM; n=6).

These results show that the protein kinase inhibitors Ro31-8220 and Ro31-7549 can reduce agonist induced constriction in small and large bronchioles as well as reducing phorbol ester induced contractions. We have also shown that smaller bronchioles are more sensitive to agonist and inhibitor concentration as well as showing a different inhibitory response profile to Ro31-8220 . This could be an indication of the relative importance of protein kinase C in these preparations which may be of therapeutic significance in diseases such as asthma.

We thank the Wellcome Trust and the Special Trustees of St Thomas' Hospital for support and Roche Products Ltd for the gifts of Ro31-8220 and Ro31-7549.

Chopra, L.C., Twort, C.H.C. and Ward, J.P.T. (1993) Am. Rev. Resp. Dis. 147, 4, A54.

81P DIFFERENTIAL PROLIFERATION OF RABBIT CULTURED TRACHEAL SMOOTH MUSCLE BY PDGF ISOFORMS: INHIBITION BY SELECTIVE INHIBITORS OF PROTEIN KINASE C AND PROTEIN TYROSINE KINASE

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The mediators and intracellular transduction events which control increases in airway smooth muscle (ASM) mass in chronic persistent asthma are poorly understood. We have investigated platelet-derived growth factor (PDGF) as a possible candidate for mediating ASM cell mitogenesis and, using reportedly selective inhibitors, explored the contribution to this response of protein kinase C (PKC)- and protein tyrosine kinase (PTK)-dependent mechanisms. PDGF comprises two distinct polypeptide chains (A and B) giving rise to three dimeric isoforms termed -AA, -BB and -AB. Target cells possess two types of PDGF receptor (PDGF-R) subunits (α and ß) which dimerise to produce functional $\alpha\alpha$, ßß or αß cell surface receptors. Current receptor models suggest that ßß receptors bind only PDGF-BB, αß receptor heterodimers bind both PDGF-BB and PDGF-AB, while $\alpha\alpha$ receptor homodimers bind all three isoforms. Thus PDGF-AA is considered to bind only to dimeric $\alpha\alpha$ receptors (Siefert *et al.*, 1989). Rabbit tracheal ASM cells were seeded ($2x10^4$cells/well) into 24 well plates containing 0.5ml Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum (FCS) for 24 hours prior to growth arrest by serum-deprivation for 72h. Growth was initiated by incubating cells with media containing PDGF isoforms in the absence or presence of 2.5%FCS and the drug under investigation. Proliferation was assessed on each day by three independent methods (Hirst *et al.*, 1992): Coomassie blue protein determination, MTT cleavage and by haemacytometry. DNA synthesis was also determined by uptake of [^{3}H]-thymidine in growth-arrested confluent cultures.

In the presence of 2.5%FCS, ASM cells proliferated in response to PDGF-BB and -AB, but not to the -AA isoform (0-60ng/ml; n=4). PDGF-induced proliferation was maximal after four days - PDGF-BB being more efficacious than PDGF-AB. In the absence of 2.5%FCS, however, the -BB and -AB isoforms (20ng/ml) *failed* to induce ASM cell proliferation (n=4), indicating the serum-dependent nature of the response. In contrast, PDGF-AB and -BB, but not -AA (20ng/ml) caused a significant ($p<0.001$) increase in DNA synthesis in the absence of 2.5%FCS. When 2.5%FCS was present all three isoforms (20ng/ml) increased DNA synthesis to a similar extent (n=5). The response to -AA being significantly ($p<0.001$) enhanced by the presence of 2.5%FCS. Prolonged exposure of confluent growth-arrested ASM cells to either the PKC inhibitor, Ro31-8220 (Davis *et al.*, 1989) or the PTK inhibitor, ST638 (Shiraishi *et al.*, 1989) in the presence of 2.5%FCS resulted in concentration-dependent inhibition of DNA synthesis induced by all PDGF isoforms. Each inhibitor was equipotent against each PDGF isoform (IC_{50}: Ro31-8220 0.3µM, n=8; ST638 15µM, n=7). Maximum inhibition of DNA synthesis occurred at 3µM Ro31-8220 and 100µM ST638. Combined incubation of Ro31-8220 (0.02-0.05µM) with ST638 (3-20µM) caused only additive inhibition of DNA synthesis induced by all three isoforms. In addition to their effects on DNA synthesis, Ro31-8220 (2µM) and ST638 (20µM) were also effective inhibitors of ASM cell proliferation induced by -AB and -BB (20ng/ml, n=4). Staining of cells with trypan blue did not occur with either of these compounds, even at higher concentrations, indicating that the cells were still viable. PDGF-AB and -BB (20ng/ml)-induced proliferation was inhibited also by a specific antibody (1µg/ml) to the ß subunit of the PDGF-R (n=4).

In conclusion, PDGF-BB and -AB isoforms are co-mitogens for rabbit tracheal ASM cells. The target receptors appear to be functionally linked to PKC- and PTK-dependent mechanisms. The ability of PDGF-AA to stimulate DNA synthesis but not proliferation suggests that $\alpha\alpha$ PDGF-R dimers are expressed by ASM cells, but are not linked to mitogenesis. The overall pattern of reactivity to the various PDGF isoforms and sensitivity to the ß subtype PDGF-R antibody suggests that rabbit ASM cell mitogenesis induced by PDGF is mediated primarily by the ß subtype PDGF-R.

We are grateful to Dr. G. Lawton (Roche Products Ltd., Welwyn Garden City, Herts.) for Ro31-8220 and to Dr. T. Shiraishi (Kanegafuchi Chemical Co., Japan) for ST638. This work was funded by the National Asthma Campaign.

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82P THE ROLE OF THE VLA-4 INTEGRIN IN A MODEL OF AIRWAY INFLAMMATION IN THE GUINEA-PIG

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In asthma an increased number of eosinophils in the airway correlates with bronchial hyperresponsiveness (Wardlaw et al., 1988). The VLA-4 integrin is expressed on eosinophils, but not on neutrophils, so adhesion via VLA-4 may provide a selective pathway for the migration of eosinophils. An antibody to the VLA-4 integrin, HP 1/2, inhibited eosinophil migration into guinea-pig skin (Weg et al., 1993), but its effects on recruitment of eosinophils to the lung is not known. Two antibodies to CD18, another leucocyte integrin, R15.7 and 6.5E, inhibited migration of eosinophils into bronchoalveolar lavage fluid (BALF) in our model of antigen-induced airway inflammation in the guinea-pig (Milne & Piper 1993). Here we report the effects of HP 1/2 in our model, on airways resistance and eosinophil content of BALF. We also assessed eosinophil content of lung tissue by measuring the activity of eosinophil peroxidase (EPO) in lung homogenates.

Guinea-pigs were sensitised to ovalbumin and challenged 2 weeks later. In separate groups of animals (n=5), 24h after challenge, airways resistance to acetylcholine (Ach, 1-30μgkg^{-1}) was measured and their lungs were lavaged (40ml buffer) and a total and differential count made of cells in BALF (Milne & Piper, 1993) or lung homogenates were prepared and EPO activity assayed (Strath et al., 1985). Antibodies, HP 1/2, R15.7, 6.5E or MOPC, an isotype control antibody, were given intravenously 1h prior to challenge and again 4h post challenge at a dose of $3mgkg^{-1}$.

Sensitisation and challenge induced bronchial hyperresponsiveness to Ach (Milne & Piper 1993) which was not affected by treatment with HP 1/2, the antibody to VLA-4. However this treatment decreased the eosinophils recovered from BALF (HP 1/2, 7.8±1.4; MOPC, 17.8±2.9 $X10^6$cells; mean±sem, $p<0.05$), although there was no change in the total number of cells recovered (HP 1/2, 30.4±4.4; MOPC, 43.4±6.8 $X10^6$ cells). In lung homogenates, the EPO activity was increased 4-fold after sensitisation and challenge; this increase was reduced by treatment with HP 1/2 but not with R15.7 or 6.5E (HP 1/2, 0.20±0.07; R15.7, 0.42±0.07; 6.5E, 0.41±0.06; MOPC, 0.60±0.09 units/mg lung protein). These results suggest that the VLA-4 integrin is involved in migration of eosinophils at the level of the vascular endothelium, while migration through the airway epithelium is largely CD18-dependent. Our results also show that bronchial hyperresponsiveness can be induced in the absence of eosinophil accumulation in airway lumen or lung tissue.

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83P EFFECTS OF ISOZYME SELECTIVE PHOSPHODIESTERASE INHIBITORS AND THEOPHYLLINE ON EOSINOPHIL INFILTRATION IN THE GUINEA-PIG LUNG

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Selective inhibitors of phosphodiesterase (PDE) isozymes have been shown to modulate inflammatory cell activity and induce relaxation of airway smooth muscle. Therefore, such inhibitors may be useful in the treatment of asthma (Beavo & Reifsnyder, 1990; Torphy & Undem, 1991). Bronchial eosinophilia and recruitment of inflammatory cells into the airway lumen are prominent features of asthma. We have investigated the effects of selective PDE isozyme inhibitors on eosinophil infiltration induced by antigen challenge or by exposure to an aerosol of platelet-activating factor (PAF) in guinea-pigs. Male Hartley guinea-pigs (300-350 g) were sensitized by exposure to an aerosol of 2 mg/ml ovalbumin (OA) solution in 0.9 % NaCl (saline) for 30 min, twice at 72 h intervals. Fifteen to 20 days later, guinea-pigs were challenged with five consecutive exposures of increasing concentrations of OA aerosols at 10 μg/ml, 100 μg/ml, 1 mg/ml, 5 mg/ml and 10 mg/ml for 15 min each respectively, or to saline alone. In another set of experiments, non-sensitized guinea-pigs were exposed to an aerosol of a 0.1 % BSA solution in saline with or without PAF (100 μg/ml). Guinea-pigs were treated orally, 24 h and 3 h before antigen challenge or PAF exposure with a non selective inhibitor: theophylline (30-100 mg/kg); type III inhibitor: Milrinone (10-30 mg/kg) and SKF 94-120 (10-30 mg/kg); type IV inhibitors: Rolipram (1-5 mg/kg) and Ro 20-1724 (3-30 mg/kg) and type V inhibitor: Zaprinast (10-30 mg/kg). The numbers of eosinophils were evaluated in the bronchoalveolar lavage (BAL) on cytospin preparation stained with May-Grünwald Giemsa, 48 H after antigen challenge or PAF exposure. In comparison to exposure of saline alone, antigen or PAF challenge significantly increased the total number of cells in BAL, as well as eosinophils. Theophylline, Rolipram and Ro 20-1724 significantly reduced the increase in eosinophil numbers in BAL, induced by antigen challenge or PAF exposure (table 1).

Table 1: % inhibition of the increase in numbers of eosinophils in BAL

Drugs	Dose (mg/kg)	Antigen challenge		PAF exposure	
Theophylline	100	-43.4%	**	-63.3%	**
	30	-29.7%	ns	-47.9%	*
Rolipram	5	-53.9%	**	-76.4%	***
	3	0%	ns	-32.5%	ns
Ro 20-1724	30	-60.6%	**	-63.2%	**
	10	-38.4%	ns	-18.9%	ns

ns: non-significant; *:$P<0.05$; **:$P<0.01$; ***: $P<0.001$ (Mann-Whitney U test) (n=5).

Milrinone (30 mg/kg), SKF 94-120 (30 mg/kg) or Zaprinast (30 mg/kg) were ineffective against eosinophilia induced by antigen challenge. Since Rolipram and Ro 20-1724 markedly inhibited the eosinophil infiltration in guinea-pig lung, these results suggest that inhibitors of PDE IV have anti-inflammatory effects in the airways and may be useful in the treatment of asthma.

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84P ELEVATION OF CYCLIC GMP OR CYCLIC AMP CONCENTRATIONS INHIBIT CONTRACTION CAUSED BY ACTIVATION OF Ca^{2+}-ENTRY OR Ca^{2+}-MOBILIZATION PATHWAYS IN BOVINE TRACHEALIS

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The possible importance of cyclic GMP as a second messenger in airway smooth muscle (ASM) has been highlighted by recent evidence suggesting that nitric oxide may be an important NANC inhibitory transmitter in ASM (Kannan & Johnson, 1992). Although it has been known for some time that cyclic GMP mediates ASM relaxation (Katsuki & Murad, 1977) and that there is a good correlation between increases in cyclic GMP concentration and relaxation in bovine tracheal smooth muscle (BTSM) (Ijioma et al, 1993) the mechanisms responsible have not been well characterised. We have therefore compared the effects of agents which elevate cyclic GMP and cyclic AMP on the spasm evoked by Ca^{2+} release from intracellular stores, Ca^{2+} influx through voltage-sensitive channels and Ca^{2+} influx through an agonist-activated, dihydropyridine-insensitive pathway in BTSM strips.

Bovine tracheal smooth muscle strips were set up for the isometric recording of tension in Krebs-Henseleit buffer (KHB) at 37°C. After 30 min equilibration tissues were repeatedly washed in Ca^{2+}-free KHB containing 2mM EGTA.

Experiments to investigate the effects of relaxants on the size of the spasm induced by 100nM methacholine were performed in Ca^{2+}-free KHB containing 2mM EGTA. Sodium nitroprusside (SNP; 10μM), glyceryl trinitrate (GTN; 10μM in the presence of superoxide dismutase (50 U/ml)) and isoprenaline (ISO; 100nM) reduced the size of the contraction relative to time-matched controls by 55.3 ± 3.5% (n=8), 71.7 ± 2.5% (n=6) and 98.6 ± 0.9% (n=6) respectively. Washing the tissue free of SNP and ISO restored the spasm to control levels but the GTN-mediated effect was only partially reversed.

The effects of SNP (10μM, n=4) and ISO (100nM, n=4) on the contraction due to Ca^{2+}-influx through voltage-sensitive Ca^{2+} channels were examined in isotonic, K^+-rich (80mM), Ca^{2+}-free KHB. Concentration-effect curves to $CaCl_2$ were constructed in the presence and absence of relaxants. The maximum $[Ca^{2+}]$ used was 12.8mM, as higher concentrations caused an irreversible contracture. SNP and ISO caused a right shift of the Ca^{2+} contraction curve (both 8 fold at 50% of response to 12.8mM $CaCl_2$ relative to control). Nifedipine (1μM, n=5) completely abolished the response to all concentrations of $CaCl_2$ tested. Curves constructed after washing to remove SNP or ISO were not significantly different from the first curve.

Murray et al (1991) have previously demonstrated that agonist-induced, Ca^{2+}-influx in ASM also occurs through a receptor-operated Ca^{2+}-influx pathway. In order to investigate the effects of SNP and ISO on this latter pathway, Ca^{2+} concentration-effect curves were constructed in Ca^{2+}-free KHB containing 100μM histamine and 1μM nifedipine. SNP (10μM) and ISO (100nM) caused 90±1.8% (n=4) and 85±2.9% (n=5) reductions in the contraction due to 1.6mM $CaCl_2$ respectively which represents a >30 fold shift to the right of the Ca^{2+} curve. Amiloride caused a concentration-dependent inhibition of the Ca^{2+} spasm (30μM caused an 88±2.7% reduction in the response to 1.6mM $CaCl_2$; n=4) suggesting that Ca^{2+} influx may be through an amiloride-sensitive, non-selective cation channel.

These data suggest that increasing either cyclic AMP or cyclic GMP in this tissue leads to an inhibition of the response to increased $[Ca^{2+}]_i$ irrespective of whether this is derived from intra- or extracellular sources. This may be occurring due to an action of the cyclic nucleotides on: (1) calcium store release; (2) Ca^{2+} influx pathways; (3) the contractile machinery of the cell or (4) a combination of these mechanisms. Further work is required to resolve the importance of each of these possible effects of the cyclic nucleotides.

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85P REGULATORY ROLE OF Ca^{2+} FOR SUPEROXIDE AND LEUKOTRIENE C_4 PRODUCTION IN HUMAN EOSINOPHILS

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Calcium (Ca^{2+}) ions are known to play a critical role in both contractile and secretory processes. Kernen et al. (1991) have previously demonstrated the Ca^{2+} dependence of eosinophil peroxidase release from human eosinophils. In this study we have investigated the role of Ca^{2+} in agonist-induced superoxide (O_2^-) and leukotriene C_4 (LTC_4) production in human eosinophils.
Human eosinophils from normal donors were assayed for O_2^- production, in the presence of cytochalasin B (5.5μM), by measuring reduction of ferricytochrome C. Supernatants from these cells were assayed for LTC_4 by radioimmunoassay. Human recombinant C5a (Sigma) (0.1 - 30nM) and fMLP (3-1000nM) caused concentration-dependent increases in O_2^- and LTC_4 production with mean EC_{50} (95% confidence limits) values of 0.9 (0.6 - 1.3)nM and 52.3 (25.7 - 106.5)nM and 2.2 (1.1 - 4.4)nM and 59.4 (37.1 - 95.3)nM, respectively (n = 9). The phospholipase C (PLC) inhibitor, U73122 (1μM) (Smith et al, 1990) caused almost complete inhibition (> 90%) of 30nM C5a and 1μM fMLP-induced O_2^- production. Chelation of extracellular Ca^{2+} using EGTA (4mM) caused marked inhibition of O_2^- release, in response to 10nM C5a (84.6 ± 16.6%; mean ± s.e.) and fMLP (63.0 ± 12.7%); LTC_4 generation was also inhibited by 96.8 ± 2.1% and 84.6 ± 18.8% respectively. Investigation of the Ca^{2+} entry pathway(s) involved in O_2^- and LTC_4 production was carried out using the putative receptor-operated Ca^{2+} channel (ROC)-blocker, SK&F 96365 (Merritt et al, 1989), the voltage-operated Ca^{2+} channel (VOC)-blocker nifedipine, and the ROC/VOC-blocker nickel (Ni^{2+}).

Table 1 — Mean IC_{50} (95% confidence limits (μM))

	O_2^- Production		LTC_4 Production		
	vs C5a (30nM)	vs fMLP (1μM)	vs C5a (30nM)	vs fMLP (1μM)	n
SK&F 96365	9.5 (3.6 - 25.1)	14.0 (8.5 - 23.0)	3.7 (1.3 - 10.9)	5.4 (2.3 - 12.7)	4
Ni^{2+}	310.2 (126.9 - 758.2)	> 3000	54.6 (4.5 - 665.4)	431.1 (37.7 - 4931.1)	4
Nifedipine	> 30.0	> 30.0	> 30.0	> 30.0	2

SK&F 96365 and Ni^{2+} caused concentration-related inhibition of both C5a- and fMLP-induced O_2^- and LTC_4 production. Nifedipine, did cause some inhibition of these responses but only at concentrations markedly higher (> 300-fold) than those required to selectively block VOCs (data not shown).
These data suggest that PLC activation and extracellular Ca^{2+} contribute to C5a and fMLP stimulation of O_2^- and LTC_4 production, in human eosinophils. The inhibitory effects observed with SK&F 96365 and Ni^{2+} but not with selective VOC-blocking concentrations of nifedipine, suggest the involvement of a ROC-mediated Ca^{2+} influx pathway.

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86P INHIBITION OF PLATELET AGGREGATION BY ACTIVATED PROTEIN C

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Protein C (PC) is a circulating zymogen which when cleaved by a thrombin-thrombomodulin complex becomes active and is known as activated Protein C (APC). APC has some anticoagulant properties due to its ability to inactivate factor Va and VIIIa. It also interacts with the plasminogen activator inhibitor, thus, enhancing tissue plasminogen activator activity and fibrinolysis. The anticoagulant properties depend on a cofactor, protein S (PS), and the association between APC and PS is species specific. Human APC only slightly increases the activated partial prothrombin time in rat plasma as compared to human plasma because rat PS does not correctly interact with human APC. However, Smirnov et all.(1990) demonstrated that human APC is able to prevent vessel occlusion in a model of arterial thrombosis in the rat. As this could be explained neither by the poor anticoagulant properties of APC in these conditions nor by its profibrinolytic properties, we decided to investigate a potential platelet antiaggregant property of human APC. Human blood was withdrawn in test tubes containing 1: 9 volume of 3.8% sodium citrate and centrifugated at 150 g to isolate the platelets and 3000 g to isolate poor platelet plasma (PPP). Platelets were suspended in PPP at a concentration of 250 000 platelets/μl and the aggregation was studied in a four channels aggregometer. APC (5 μg/ml) was added 3 minutes before or after agonist addition. Results are given as percentage of aggregation followed by aggregation slope and the number of replicates. (adenosine-5'-triphosphate, ADP)

	ADP 20 μM			Collagen 190 μg/ml			Arachidonic acid 0.5 mg/ml			Ristocetin 1.5 mg/ml		
Control	72 ± 3	38 ± 1	(9)	83 ± 1	28 ± 5	(6)	64 ± 3	23 ± 2	(11)	80 ± 6	37 ± 3	(6)
APC 3 min before	17 ± 2	17 ± 1	(7)	67 ± 6	11 ± 2	(4)	5 ± 1	5 ± 1	(4)	45 ± 2	22 ± 2	(4)
APC 3 min after	51 ± 3	37 ± 2	(7)	67 ± 4	17 ± 1	(4)	21 ± 1	20 ± 1	(4)	38 ± 5	32 ± 1	(4)

A weaker aggregation or a disaggregation were observed in the APC treated groups. Following an analysis of variance, aggregations in the APC treated groups were significantly different from control whatever was the agonist (Fisher post-hoc tests, $p \leq 0.001$ in all cases). When treated with diisofluorophosphate 5 mM, which forms a stable complex with the anticoagulant active serine site of APC, the aggregation provoked by ADP addition 3 minutes after APC treatment reached -6 ± 2% compared to 56 ± 5% in the control (n=5), proving that this site is not involved in the observed effect. Platelets cyclic nucleotide contents were not different between control and platelets treated with APC. This suggested that APC has an effect much downstream in the aggregation pathway, directly at the IIb-IIIa receptor level. In an in vitro study, using radiolabelled APC and isolated platelet IIb-IIIa receptors, a two dimensions electrophoresis followed by an autoradiography showed a strong association between the IIb-IIIa peak and radiolabelled APC. This confirms that APC may inhibit platelets aggregation through an interaction with the IIb-IIIa receptor and may disturb the setting up of fibrinogen bridges between platelets. After PC cleavage, a RGD (Arg-Gly-Asp) sequence is exposed at the end of the proteic chain of the so generated APC. This could explain the interaction with IIb-IIIa receptors. In conclusion, besides its anticoagulant and profibrinolytic properties, human APC also has antiplatelet properties. Thus, APC may be proposed as an adjunct treatment during fibrinolysis and angioplasty.

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87P REGULATION OF PHOSPHATIDYLINOSITOL 3-KINASE ACTIVITY IN HUMAN PLATELETS: ASSOCIATION OF THE ENZYME WITH VARIOUS TYROSINE-PHOSPHORYLATED SUBSTRATES

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In recent years, interest in Phosphatidylinositol 3-kinase (PI 3-kinase) has fueled because of the suggestion that this metabolic pathway may harbor new second messenger(s). PI 3-kinase catalyzes the production of a family of phosphoinositides (PI-3P, PI-3,4P_2, and PI-3,4,5P_3) with phosphates at the D-3 position on the inositol ring. Studies have shown that PI 3-kinase stimulation is a common sequel to protein-tyrosine kinase (PTK) activation and appears to be essential for PTK mediated mitogenic responses and oncogenic transformations. Recent studies demonstrating the occurrence of 3-phosphorylated phosphoinositides (3-PPI) in non-proliferating terminally differentiated cells, such as platelets, suggest the involvement of these novel molecules in processes distinct from growth control.

The involvement of protein-tyrosine (Pr-Y) phosphorylation in the regulation of PI 3-kinase activity is suggested in the literature. Since many physiological agonists activate PTKs in human platelets, we explored the potential role of Pr-Y phosphorylation in the regulation of PI 3-kinase activity during platelet activation. *In vitro* PI 3-kinase assays were performed on anti-phosphotyrosine [anti-Tyr(P)] antibody immune complexes of detergent-soluble lysates from collagen (1.8μg/ml), thrombin (1U/ml), thromboxane A_2 mimetic U46619 (5μM), Ca^{2+} ionophore A23187 (0.5mM) or phorbol-12-myristate-13-acetate (PMA) (100nM) stimulated platelets. A time dependent increase in PI 3-kinase activity was observed in anti-Tyr(P) antibody immune complexes of platelets stimulated by thrombin, collagen, U46619 and A23187. In contrast, PMA stimulated platelets showed very small increase in the PI 3-kinase activity. The presence of increased PI 3-kinase activity in anti-Tyr(P) antibody immune complexes of stimulated platelets suggests one or more of the following possibilities: 1) increased tyrosine phosphorylation of PI 3-kinase, 2) increased association of PI 3-kinase with tyrosine phosphorylated protein(s), or 3) increased specific activity of PI 3-kinase molecules that are already tyrosine phosphorylated. *In vitro* kinase assays on anti-p85 antibody immune complexes of detergent-soluble lysates of stimulated platelets showed no change in the specific activity of PI 3-kinase. The anti-Tyr(P) immunoblot analysis and phosphoamino acid analysis of anti-p85 antibody immune complexes showed that the p85 or p110 subunits of platelet PI 3-kinase were not phosphorylated on tyrosine in stimulated platelets and controls. Instead, the anti-Tyr(P) immunoblot revealed the presence of phosphotyrosyl proteins co-immunoprecipitating with p85 subunit of PI 3-kinase. These results indicate that direct tyrosine phosphorylation of PI 3-kinase is not required for its activation in human platelets and stimulus-dependent association of PI 3-kinase with other tyrosine-phosphorylated proteins may play an important role in regulation of its activity during platelet activation.

88P CALCIUM, ATP AND H_2O_2 INHIBITION OF STIMULATED HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS

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Exogenous or endogenous H_2O_2 (a reactive oxidative released by activated neutrophils and other inflammatory cells) is capable of altering cellular functions and might play a role in immunosuppression and inflammation. Histamine is involved in both processses. The aims of the present work were to study (1) the effects of H_2O_2 and t-butylhydroperoxide (tbOOH, a short chain analogue of lipid hydroperoxides similar to those generated during oxidative stress) on histamine release (HR) induced by anti-IgE, 48/80, NaF and A23187, four stimulants each with a different mode of action and (2) whether H_2O_2 or tbOOH would affect cell viability, cell membrane stability, intracellular levels of Ca^{2+} and ATP which have been shown to be affected by H_2O_2 in other systems (Vercelloti et al., 1991).

Rat peritoneal mast cells (RPMC) were obtained by direct lavage. For anti-IgE experiments, the RPMC were from animals sensitized with *Nippostrongylus brasiliensis*. Trypan blue extrusion was used to assess cell viability. ATP was determined using a linked assay with phosphoglyceric phosphokinase and glyceraldehyde phosphate dehydrogenase (Sigma). Chlorotetracycline (CTC) was used to measure intracellularly bound Ca^{2+} (Naccache et al., 1979).

	H_2O_2 (mM)			tbOOH (mM)			
	0.01	0.1	1.0	0.01	0.1	1.0	The table shows percentage inhibition (mean±s.e.m., N=
Anti-IgE (1/300 dilution)	75.2±2.4	93.6±3.6	95.2±5.2	49.8±3.5	74.6±4.3	82.2±3.1	of HR by H_2O_2 and tbOOH.Cells were preincubated
48/80 (0.5 ug/ml)	28.6±2.1	81.5±3.5	92.2±4.1	30.1±2.1	62.4±3.2	92.2±1.4	with the compounds for 30 min and then for a
A23187 (10 uM)	19.6±2.3	49.0±3.2	88.7±2.1	50.0±3.6	89.2±3.2	91.2±4.1	further 10-30min following stimulation.
NaF (10 mM)	25.3±1.2	78.2±2.5	89.2±3.1	19.3±0.5	88.8±1.7	93.0±2.0	HR for all stimulants was 48-64 %.

Both H_2O_2 and tbOOH are seen to produce a marked and dose-related inhibition of HR induced by all stimulants used. DL-propranolol, which has cell membrane stabilizing actions, had no effect on the inhibition. However, catalase (a scavenger for H_2O_2, 10 U/ml) completely reversed the inhibitory effects of H_2O_2 and tbOOH for all four stimulants. Both compounds caused a marked decrease in intracellularly bound Ca^{2+} ions as assessed by a reduction in CTC fluorescence (H_2O_2, 1mM, 45.3±3.6 %, N=3; tbOOH, 1mM, 48.4±3.1 %, N=3). H_2O_2 and tbOOH (1mM) produced a slight reduction in total ATP (12.2±2.1 and 16.0±4.2 %, respectively, N=3) and cell viability was not changed by either treatment. In total, these data show that H_2O_2 and tbOOH can profoundly affect mast cell function possibly by interfering with calcium homeostasis.

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89P STIMULATION OF ANGIOGENESIS BY THYMIDINE PHOSPHORYLASE AND PLATELET-DERIVED ENDOTHELIAL GROWTH FACTOR

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Platelet-derived endothelial cell growth factor (PD-ECGF) is an angiogenic factor with an unknown mechanism of action. *In vitro* studies showed that PD-ECGF stimulates [^{3}H]thymidine uptake in endothelial cells without causing them to proliferate (Moghaddam & Bicknell, 1992). It was therefore suggested that PD-ECGF may stimulate angiogenesis indirectly through its thymidine phosphorylase (TP) activity (Moghaddam & Bicknell, 1992; Usuki, *et al.*, 1992). To test the hypothesis that the *in vivo* angiogenic effect of PD-ECGF is related to its intrinsic TP activity, we carried out comparative studies on the ability of *Escherichia coli* recombinant TP and *E. coli* recombinant PD-ECGF to elicit angiogenesis *in vivo*.

Sterile polyether sponges with attached cannulae were implanted subcutaneously in male Wistar rats (180-200g) and neovascularisation was assessed by using ^{133}Xe clearance to measure blood flow through the implants every two days over a period of 14 days. Test substances (50 µl) were applied to the sponges through the cannulae daily 24h before ^{133}Xe measurement. The effects of test substances on angiogenesis were confirmed histologically.

Daily administration of 10 pmol PD-ECGF into the sponges caused an intense neovascularisation. The 6 min ^{133}Xe clearance values obtained on Day 6, 8, 10 for the PD-ECGF group and the control group were 20.8±1.5, 31.4±2.5, 39.2±1.9 and 14.5±0.4, 19.6±0.5, 31.1±2.0 %, respectively ($P < 0.01$, $n = 6$). This PD-ECGF-induced neovascular response was inhibited by co-administration of a rabbit polyclonal neutralising antiserum to PD-ECGF (PD-ECGF$_{AS}$; 25 µg day^{-1}). The ^{133}Xe clearance values on Day 6, 8, 10 for the co-administration group was 14.8±1.5, 19.5±1.0, 31.0±1.5 % ($P < 0.05$, $n = 6$).

Daily administration of 10 pmol *E. coli* TP into the sponges also stimulated angiogenesis. The 6 min ^{133}Xe clearance values obtained on Day 6, 8, 10 for the TP group and the control group were 20.5±0.7, 29.2±1.3, 39.9±0.6 and 14.5±0.4, 19.6±0.5, 31.1±2.0 %, respectively ($P < 0.01$, $n = 6$). This TP-induced neovascular response was partially inhibited by co-administration of PD-ECGF$_{AS}$ (25 µg day^{-1}). The ^{133}Xe clearance values on Day 6, 8, 10 for co-administration group was 16.6±1.0, 23.7±1.9, 32.7±1.7 % ($P < 0.05$ for Day 6 & Day 10, $n = 6$). Lower doses of PD-ECGF (0.1 and 1 pmol) or TP (1 pmol) produced no apparent effect on sponge-induced neovascularisation.

Histological studies of sponge sections showed that PD-ECGF$_{AS}$ suppressed the massive cellular infiltration and intense angiogenesis in the PD-ECGF- and TP-treated sponges. The specificity of PD-ECGF$_{AS}$ was demonstrated by its lack of effect on sponge-induced neovascularisation or the neovascular response elicited by daily doses of 100 ng bFGF.

Thus, our data strongly suggest that thymidine phosphorylase activity is an integral part of the angiogenic activity of the PD-ECGF molecule. Further studies with PD-ECGF mutants having different enzyme activity will help elucidate the role of thymidine phosphorylase in angiogenesis.

This work is supported by the Wellcome Trust.

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90P DIFFERENTIAL EFFECTS OF INTRA-RAPHE INJECTIONS OF SENKTIDE ON 5-HT OVERFLOW IN THE HIPPOCAMPUS OF CONSCIOUS FREELY-MOVING RATS

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Tachykinin NK$_3$ receptors are found in high density in median (MRN) but not dorsal raphe nuclei (DRN) (Stoessl and Hill 1990). Autoradiographic studies suggest that 5-hydroxytryptamine (5-HT)-secreting neurones in MRN innervate both dorsal (DH) and ventral hippocampus (VH) whereas DRN projections to hippocampus innervate VH only (Azmitia and Segal 1978). This study examined the differential effects of the NK$_3$ agonist, senktide, on 5-HT projections from these two nuclei. Microdialysis probes (Benwell & Balfour 1992), located in either DH (AP:-5.2mm , L:2.9mm relative to Bregma, V:3.1mm from brain surface) or VH (AP:-5.8mm, L:4.9mm, V:7.0mm) and guide cannulae, targeted at either MRN (AP:-7.8mm, L:2.0mm, V:6.2mm at 14.5° to vertical) or DRN (AP:-7.8mm, L:3.0mm, V:4.25mm at 17.5° to vertical), were implanted into the brains of male Sprague-Dawley rats. On the following day, the dialysis probes were perfused with Ringer solution (1.7µ l/min.). Following a 60 minute equilibration period, 3 x 15 min. baseline samples of dialysate were collected before drug solution or vehicle (0.3% DMSO in saline (senktide expt); saline (8OHDPAT expt)) was injected (0.5µl/2min.) into MRN or DRN via micro-cannulae and a further 8 x 15 min. samples were collected. Samples were analysed by HPLC with electrochemical detection. Data are presented as percentages of the mean 5-HT concentration in the 3 baseline samples and are expressed as means ± SEM. Mean 5-HT overflow per 15 min. in the DH following injection of vehicle was 85±4% (n=8) of baseline; in VH it was 77±5% (n=8). Intra-MRN injections of senktide increased (drug x time $F(14,84)=3.6;P<0.001$) 5-HT overflow in DH to a peak response of 226±32% (1pmole; n=4) and 255±33% (10pmoles; n=5). Intra-DRN injections of senktide increased (drug x time $F(14,63)=2.6;P<0.01$) 5-HT overflow in DH to a peak response of 123±23% (1pmole; n=4) and 151+15% (10pmoles; n=4). The peak response to intra-MRN injections of senktide was observed during the first hour post-injection; peak responses to intra-DRN injections occurred during the second hour. Intra-DRN injections of senktide had no effects on 5-HT overflow in VH, when compared with control, but increased overflow (drug x time $F(14,84)=2.5;P<0.01$) in VH was observed following intra-MRN injections (peak response: 176±14% and 137±19% for 1 (n=5) and 10pmoles (n=5) respectively). These peaks occurred during the second hour post-injection. 5-HT overflow in VH, following an intra-DRN injection of 8OHDPAT (7.6nmoles) was reduced (drug $F(1,9)=150;P<0.001$) to a mean level of 30±3% (n=6) whereas 5-HT overflow in DH (117±4%; n=5) was higher (drug $F(1,7)=10.;p<0.05$) than control values post-injection. Intra-MRN injections decreased (drug $F(1,8)=15.3;P<0.01$) 5-HT overflow in VH to a mean value of 53±4% (n=5) and also decreased (drug x time $F(7,56)=2.5;P<0.5$) 5-HT overflow in DH to 60±6% (n=4) when compared with control (86±4%; n=6) during the first hour post-injection but not during the second hour. The data suggest that the MRN innervates the areas of both DH and VH dialysed in these experiments whereas the serotonergic fibres from the DRN project to VH but not DH. They also suggest that NK$_3$ receptors are expressed on the serotonergic cells which project from MRN to DH. The mechanisms underlying the delayed changes in 5-HT overflow in DH and VH remain to be established.

FLG is supported by a postgraduate studentship from Glaxo Group Research

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91P A PHARMACOLOGICAL PROFILE OF WAY-100635, A POTENT AND SELECTIVE 5-HT_{1A} RECEPTOR ANTAGONIST

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Although extensive research efforts have been focussed on the 5-HT_{1A} receptor subtype, further validatory work has been hampered by the lack of highly selective 5-HT_{1A} receptor antagonists. Until recently, ligands used as 5-HT_{1A} receptor antagonists were either non-selective or 5-HT_{1A} receptor partial agonists (for review, see Cliffe et al., 1993). To be designated as a 5-HT_{1A} receptor antagonist, the ligand must display antagonist activity, without evoking an agonist response, at both presynaptic, somatodendritic 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} receptors. Here we report the pharmacological properties of a novel, potent compound which meets these requirements, i.e. WAY-100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride), an analogue of the recently-reported antagonist, WAY-100135 (Fletcher et al., 1993).

Standard radioligand binding techniques were used to determine the affinity of WAY-100635 at 5-HT_{1A} and other CNS receptor sites. In functional studies, the effects of WAY-100635 alone, and on the agonist responses evoked by 8-OH-DPAT were examined on dorsal raphe neuronal firing in anaesthetised rats, induction of the '5-HT syndrome' (flat posture, forepaw treading and hyperlocomotion) in rats and induction of hypothermia in the mouse and rat, as described previously (Fletcher et al., 1993).

The IC_{50} of WAY-100635 for displacement of specific [^{3}H]-8-OH-DPAT binding to rat hippocampal membranes was 2 nM and the corresponding values at all other binding sites studied were at least 100-fold greater than the IC_{50} at 5-HT_{1A} sites. At a dose of 100 µg/kg i.v., WAY-100635 alone had no significant effect on raphe neuronal firing but shifted the ID_{50} of 8-OH-DPAT to inhibit cell firing from 1.9 µg/kg i.v. to >10 µg/kg i.v. Administered alone, up to a toxic dose (10 mg/kg i.v.), WAY-100635 did not evoke any components of the '5-HT syndrome' in the rat. However, at doses of 0.003-3.0 mg/kg s.c., WAY-100635 significantly increased the ED_{50} of 8-OH-DPAT to induce the 5-HT behavioural syndrome. In addition, WAY-100635 (0.003-0.1 mg/kg s.c.) also blocked the hypothermia induced by 8-OH-DPAT (0.25 mg/kg s.c.) in the rat, with an ID_{50} of 0.01 (0.004-0.027) mg/kg s.c. WAY-100635 (0.001-0.1 mg/kg s.c.) was a highly potent antagonist of 5-HT_{1A} receptor-mediated responses in other species, i.e. in the guinea-pig, the ID_{50} for antagonism of the 5-HT syndrome response to 8-OH-DPAT (0.5 mg/kg s.c.) was 0.01 (0.003-0.025) mg/kg s.c., and in the mouse, the ID_{50} for blockade of 8-OH-DPAT (0.5 mg/kg s.c.) - induced hypothermia was 0.01 (0.004-0.022) mg/kg s.c.

WAY-100635 represents a considerable advance over recently described prototype 5-HT_{1A} receptor antagonists in that it is considerably more potent and selective than any compound used previously as a 5-HT_{1A} receptor antagonist. In addition to blocking several postsynaptic 5-HT_{1A} receptor-mediated responses, it has also been demonstrated to block a range of responses mediated by somatodendritic 5-HT_{1A} receptors (see also Gurling et al., Critchley et al. and Hartley et al., this meeting).

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92P SINGLE-UNIT RECORDINGS FROM FREELY-MOVING ANIMALS PROVIDE EVIDENCE THAT WAY-100635, BUT NOT (S)-WAY-100135, BLOCKS THE ACTION OF ENDOGENOUS SEROTONIN AT THE 5-HT AUTORECEPTOR

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Somatodendritic 5-HT_{1A} autoreceptors play an important role in regulating the discharge activity of central serotonergic neurones. Recently we have shown that blockade of these receptors by spiperone increases the firing rate of central serotonergic neurones in the awake animal (Jacobs & Fornal, 1991). This suggests that serotonin autoreceptors exert a tonic inhibitory influence on the activity of serotonergic neurones under physiological conditions. Recently, (S)-WAY-100135 and WAY-100635 have been described as potent and selective antagonists at 5-HT_{1A} receptors (Fletcher et al., 1993, 1994 ; Critchley et al., 1994 ; Gurling et al., 1994) The present study examined the effects of these novel compounds on the activity of serotonergic neurones recorded in freely moving animals.

Adult cats (2.5-5.0 kg) were implanted with nichrome microwires for recording single-unit activity in the dorsal raphe nucleus. Cells were identified as serotonergic based on previously established criteria (Jacobs & Fornal, 1991). Drugs were administered remotely via an indwelling jugular catheter.

Administration of (S)-WAY-100135 (0.1, 0.5 and 1 mg/kg) produced a modest suppression of serotonergic unit activity lasting approximately 15 to 30 min. The mean (± SEM) percent decrease in firing rate produced by (S)-WAY-100135 was 36 ± 10 at 0.1 mg/kg (n=6), 41 ± 10 at 0.5 mg/kg (n=9) and 43 ± 7 at 1 mg/kg (n=5). In contrast to (S)-WAY-100135, administration of WAY-100635 (0.1 and 0.5 mg/kg) produced a significant increase in serotonergic neuronal activity. The mean percent increase in basal firing rate produced by WAY-100635 was 55 ± 21 at 0.1 mg/kg (n=3) and 65 ± 15 at 0.5 mg/kg (n=7). The effect of WAY-100635 was evident during wakefulness (a period of relatively high neuronal activity), but not during sleep (a period of relatively low neuronal activity). In addition, we examined the ability of these compounds to block the inhibitory action of the specific 5-HT_{1A} agonist 8-OH-DPAT on serotonergic neuronal activity. Preliminary results indicate that both (S)-WAY-100135 and WAY-100635 block this action of 8-OH-DPAT, although the latter compound appears to be more effective.

Overall, these data suggest that WAY-100635 acts as a selective 5-HT_{1A} autoreceptor antagonist, whereas (S)-WAY-100135 may have additional properties. These data also support the hypothesis that autoreceptor-mediated feedback inhibition operates under conditions when cells are firing at a relatively high rate (i.e. during quiet waking and arousal).

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93P EFFECT OF THE PUTATIVE 5-HT$_{1A}$ ANTAGONIST WAY100635 ON 5-HT NEURONAL FIRING IN THE GUINEA-PIG DORSAL RAPHÉ NUCLEUS

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The selective 5-HT1A agonist 8-OHDPAT (8-hydroxy-2-(di-n-propylamino)tetralin) produces a reversible inhibition of neuronal firing by an agonist action at the pre-synaptic 5-HT1A somatodendritic autoreceptor in the rat (Sprouse and Aghajanian 1987) and guinea-pig dorsal raphe nucleus (DRN) (Mundey et al., 1992). The 5-HT1A antagonist WAY100135 (N-tert-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2phenylpropanamide dihydrochloride) (Fletcher et al., 1993) blocks this effect in the guinea-pig whilst having no effect on basal neuronal activity (Mundey et al., in press). WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(pyridinyl) cyclohexanecarboxamide trichloride is a more potent analogue of WAY100135 and the present study has used *in vivo* electrophysiology in the guinea-pig DRN to evaluate the effects of WAY100635 on 5-HT neuronal firing.

Male Dunkin-Hartley guinea-pigs (320-450g) were anaesthetised with urethane (1.3g kg^{-1} i.p.) and the jugular vein cannulated for i.v. administration of drugs. Single barrelled glass electrodes, filled with 2M NaCl containing 2% pontamine sky blue dye, were implanted into the DRN using stereotaxic coordinates taken from lambda (A +0.7-1.0; L 0.0; V -6.5-7.5mm). 5-HT neurones were identified by their slow, regular firing pattern (0.5-4.0 spikes sec^{-1}.) and their reversible inhibition (83±15%, n=10, $p<0.05$) by 8-OHDPAT (10μg kg^{-1} i.v.). WAY100635 (0.125-0.5mg/kg i.v.) produced a dose-related increase in the basal firing rate of 8-OHDPAT sensitive neurones (upto 45% over basal, n=8) for between 10-30 minutes after administration. WAY100635 (0.125mg/kg i.v.) completely blocked 8-OHDPAT induced inhibition of neuronal firing (n=8) and furthermore could restart the firing of neurones already inhibited by 8-OHDPAT (10μg/kg i.v.,n=6). WAY100635 produced a rightward shift in the dose related inhibition of neuronal firing by 8-OHDPAT (5-100μg/kg i.v.) (ED_{50} increased from 6.6 ± 1.5 to 34.8 ± 5.6μg/kg i.v., n=6) but did not effect the maximum inhibition elicited by 8-OHDPAT. This provides evidence of a competitive interaction between 8-OHDPAT and WAY100635 for the 5-HT1A somatodendritic autoreceptors in the dorsal raphe nucleus of the guinea-pig. These results demonstrate that WAY100635 is a potent 5-HT1A antagonist at the presynaptic sites and the increase in basal rate of neuronal firing, suggests that there is a 5-HT1A mediated serotonergic inhibitory tone in the dorsal raphe nucleus of the guinea-pig.

M.K.M. is a SERC CASE award student in conjunction with Wyeth Research (U.K.) Ltd.
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94P SYSTEMIC FLESINOXAN DEPRESSES HIPPOCAMPAL 5-HT RELEASE IN THE FREELY-MOVING GUINEA-PIG

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Flesinoxan is a selective 5-HT$_{1A}$ receptor agonist acting at central sites (Wouters et al., 1988). We have examined the effect of flesinoxan on 5-HT and 5-HIAA levels measured in the hippocampus of the freely moving guinea pig and compared the consistency of flesinoxan-induced effects within a single animal when reused at one weekly intervals.

Male Dunkin-Hartley guinea-pigs (~400 g) were used. Under anaesthetic a guide cannula (CMA-12) was stereotaxically implanted with its tip adjacent to the dorsal hippocampus. The animals were allowed one week to recover from surgery and were introduced to the recording apparatus. Each afternoon prior to flesinoxan administration a microdialysis probe (CMA-12) was introduced into the hippocampus and perfused with dialysis fluid (2 µl.min^{-1}), containing (in mM) NaCl 147, KCl 4, $CaCl_2$ 1.5, $MgCl_2$ 0.7 and fluvoxamine 0.01. Sample collection (20 min) started the following morning and continued throughout the duration of the experiment. Samples were assayed for both 5-HT and 5-HIAA (HPLC with ECD). Following a 2 h baseline period flesinoxan was administered either i.p. (1-10 mg.kg^{-1}) or via the dialysis probe (1 µM). Samples were collected for a further 4 h and then tetrodotoxin (TTX, 1 µM) was perfused through the probe. The mean values of 5-HT and 5-HIAA of the first 6 samples were taken as the baseline values (100%) and drug effects expressed relative to these values. Results are given as mean ± SEM (n). In this study 24 guinea pigs were examined, each animal was used between 1 and 4 times.

The baseline values for 5-HT and 5-HIAA (pg. 20 min^{-1}) were 5.9 ±0.4 (n=37) and 239 ±15 (n=37) respectively. TTX reduced 5-HT levels to 8.8% ±1.0 (n=37) within 1 hour of perfusion. 5-HIAA levels were reduced to approximately 80%. Flesinoxan administered via the probe (1 µM, n=3) did not alter measured levels of 5-HT or 5-HIAA although in one of these experiments 5-carboxamidotryptamine (0.1 µM) depressed 5-HT levels to approximately 60%. Following i.p. injection flesinoxan induced a long lasting dose-dependent depression of the measured 5-HT level to 58.8% ±10.3 (n=8) at 1 mg.kg^{-1}, 27.8% ±5.2 (n=15) at 3 mg.kg^{-1} and 17.1 ±8.6 (n=4) at 10 mg.kg^{-1}. The depressant effect of flesinoxan was evident within 20 min and maximum within 80 min. 5-HIAA levels were maximally depressed to approximately 70% within 180 min. In three animals flesinoxan 3 mg.kg^{-1} was administered at weekly intervals. The flesinoxan induced effect was assessed by integrating the time-effect curve over a 4 h period. The variability in response for individual animals on three separate occasions was not greater than between three different animals on any particular day. This would suggest that reuse of animals under certain conditions is acceptable.

In addition to reporting the effect of the 5-HT$_{1A}$ receptor agonist flesinoxan on hippocampal 5-HT and 5-HIAA levels in the freely moving guinea pig this work suggests a useful method for reducing the number of animals used in microdialysis experiments.

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95P DIURNAL VARIATION IN NERVE TERMINAL 5-HT AUTORECEPTOR FUNCTION *IN VIVO*: EFFECTS OF ANTIDEPRESSANT TREATMENT

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Disturbed biological clock function is implicated in the aetiology of depression (Hallonquist et al., 1986). 5-Hydroxytryptamine (5-HT) release in the rat hypothalamus (the site of the biological clock) has a circadian rhythm and can be altered by 5-HT1B autoreceptor activation (Martin, 1991). Since many antidepressants alter 5-HT function and because 5-HT may be involved in biological clock control, we have determined whether 5-HT1B function in rat hypothalamus has a diurnal variation and is altered by antidepressant treatment.

Male Wistar rats (240-260 g; Olac, Bicester) housed under a 12:12h light:dark cycle (lights on 07:00) were given 21 daily i.p. injections of saline, paroxetine (10 mg/kg) or desipramine (10 mg/kg). Twenty-four hours after the last dose, the rats were anaesthetised with chloral hydrate (600 mg/kg ip and 30 mg as required) and concentric microdialysis probes (Carnegie Medecin), perfused (1 µl/min) with an artificial cerebrospinal fluid containing the selective 5-HT reuptake inhibitor citalopram (1 µM), were stereotaxically implanted into the anterior hypothalamus. Perfusates were collected every 15 min and analysed for 5-HT by HPLC-ECD. After a 2 h stabilisation period, RU24969 (5 µM, Martin, 1991) was administered via the perfusion fluid in a 15 min pulse at either mid-light period (ML) or end-light period (EL). Basal release of 5-HT was 38.7 ± 0.3 (n=4) and 28.4 ± 0.1 (n=6) fmoles per 15 min sample at ML and EL respectively. Antidepressant treatment had no significant effects on basal release except at EL, when desipramine increased basal 5-HT by 45% ($p<0.05$). Dialysate 5-HT was significantly decreased by RU24969 at ML and EL but to a greater extent at EL (Table 1). Paroxetine or desipramine treatment significantly reversed the inhibition of 5-HT release induced by RU24969 (Table 1).

Table 1 The effect of RU24969 on 5-HT release in the rat anterior hypothalamus after chronic antidepressant treatment.

TREATMENT	n	0 min	15 min	30 min	45 min	60 min	75 min	90 min	105 min
ML Control	4	98±2	71±2	52±1#	54±2#	55±2#	49±2#	55±1#	67±3
ML Paroxetine	5	98±1	79±2	70±1	65±2	62±3	54±3	89±7**	129±4*
ML Desipramine	4	97±1	81±2	71±2	62±1	53±2	54±3	98±4**	131±6*
EL Control	6	98±1	83±7	72±7	53±5#	30±2#	17±2#	7±1#	8±1#
EL Paroxetine	4	101±1	70±2	48±4	44±3	43±2**	48±3**	49±1**	42±1**
EL Desipramine	4	98±1	69±2	65±2	71±3*	86±3**	88±3**	92±1**	92±1**

Values are mean ± s.e.mean percentage of mean pre-intervention dialysate 5-HT concentration. RU24969 infused between 0 &15 min. Statistical analysis: one-way ANOVA followed by Studentised range test: #P<0.01 vs. no infusion, *P < 0.05, **P<0.01 vs. Control.

These data demonstrate that hypothalamic 5-HT1B autoreceptor function varies with time of day. In addition, they show that antidepressants down-regulate 5-HT1B function at ML and EL, but have a greater effect at EL, when the activity of this receptor is highest. Thus, the data suggest that antidepressant efficacy may involve modification of the 5-HT input to the biological clock and thereby, circadian rhythm control.

TJOS is supported by the SERC and Boots Pharmaceuticals

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96P THE HUMAN 5-HT$_{1D\alpha}$ RECEPTOR IS POSITIVELY COUPLED TO ADENYLYL CYCLASE IN TRANSFECTED CHO CELLS

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There are at least two subtypes of the human 5-HT1D receptor, 5-HT1Dα and 5-HT1Dβ, both of which are believed to be negatively coupled to adenylyl cyclase (Weinshank et al., 1992). We now provide evidence that the human 5-HT1Dα receptor, expressed in Chinese hamster ovary (CHO) cells is positively coupled to this enzyme.

CHO cells stably expressing human 5-HT1Dα or 5-HT1Dβ receptors (B_{max}=5.67 and 1.16 pmoles/mg protein respectively: data obtained from [^{3}H]5-HT receptor binding studies) were grown to confluence in 80cm^2 flasks and harvested by centrifugation. Cells (2 x 10^5) were incubated with isobutylmethylxanthine (500 µM) in the absence or presence of forskolin, with or without test compounds and the reaction terminated by the addition of 3M perchloric acid. Following extraction, the accumulated cAMP was measured by radioimmunoassay.

In the parental CHO cell line forskolin (10 µM) stimulated cAMP accumulation but 5-HT had no significant effect on basal or stimulated levels. In the 5-HT1Dβ cell line forskolin (10 µM) induced a 9-fold increase in cAMP levels (Table 1). 5-HT had no effect on basal levels but inhibited forskolin-stimulated cAMP accumulation in a concentration dependent manner (pEC_{50}=7.8 ± 0.04;E_{max}=70 ± 5%). In 5-HT1Dα clones, cAMP levels were increased 8-fold by forskolin (100 µM) and this stimulation was enhanced in a concentration dependent manner by 5-HT (pEC_{50}=7.7 ± 0.1;E_{max}=260 ± 40% of forskolin control). The pEC_{50}s for 5-HT obtained with the two clones were comparable with the pK_is obtained in binding studies (Weinshank et al., 1992). Methiothepin (1 µM) shifted to the right both the 5-HT inhibitory response curve in 5-HT1Dβ clones (apparent pA_2 = 8.9 ± 0.1) and the 5-HT stimulatory effect in 5-HT1Dα clones (apparent pA_2 = 9.1 ± 0.1). Methiothepin (1 µM) alone had no significant effect on basal cAMP levels in either clone.

Table 1

Cell Line	cAMP Levels (pmol/400,000 cells ± S.E.M.)			
	Basal	+ forskolin	+ forskolin +5-HT (1µM)	+ 5-HT [1µM]
Parental	8.2 ± 2.6 (n=7)	25.9 ± 8.0 (n=5)	17.3 ± 5.8 (n=4)	14.7 ± 3.8 (n=3)
5-HT1Dα	6.3 ± 0.5 (n=12)	49.8 ± 12.9 (n=3)	119.5 ± 18.3 (n=3)	16.8 ± 2.5 (n=8)
5-HT1Dβ	5.3 ± 0.4 (n=9)	46.8 ± 7.3 (n=7)	14.2 ± 1.6 (n=6)	5.3 ± 0.7 (n=3)

We conclude that CHO cells transfected with the 5-HT1Dα gene produce agonist stimulation of cAMP accumulation, under the conditions employed. This is in agreement with Maenhaut et al (1991) who reported a 5-HT-induced stimulation of cAMP levels in three different cell lines expressing the RDC4 gene, the dog homologue of the 5-HT1Dα receptor.

We are extremely grateful to D. Pritchett and C Mannix for providing the cell lines.

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97P BIOCHEMICAL AND FUNCTIONAL CHARACTERISATION OF THE CLONED HUMAN 5-HT_{2A} RECEPTOR EXPRESSED IN THE HUMAN NEUROBLASTOMA CELL LINE SH-SY5Y

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Activation of 5-HT_{2A} receptors stimulates phosphoinositide (PI) hydrolysis with the formation of inositol trisphosphate and diacylglycerol and a subsequent rise in intracellular calcium (Leysen, 1992). The electrophysiology of the 5-HT_{2A} receptor in rat central neurones has also been characterised where it induces neuronal depolarisation (North & Uchimura, 1989). Expression of the human 5-HT_{2A} receptor cDNA in a cell line would allow further investigation into the functional consequences of 5-HT_{2A} receptor activation. We have therefore investigated the binding characteristics, inositol phosphate formation, calcium elevation and electrophysiological responses of the cloned human 5-HT_{2A} receptor expressed in the human neuroblastoma cell line SH-SY5Y.

SH-SY5Y/5-HT_{2A} stable clones were isolated on the basis of resistance to geneticin encoded by the mammalian expression vector pCDN containing the human 5-HT_{2A} receptor cDNA. A single clone selected for further study bound [^{3}H]ketanserin with high affinity (Kd = 0.7 nM) and demonstrated high expresion levels (Bmax = 550 fmol/mg protein). Inhibition of [^{3}H]ketanserin binding showed the expected profile for the 5-HT_{2A} receptor with potency decreasing in the order: spiperone > ketanserin > mianserin > mesulergine. The transfected 5-HT_{2A} receptor demonstrated functional coupling to the PI second messenger system. Production of inositol phosphates ([^{3}H]IP_X) was increased by 5-HT in a concentration-dependent manner (pEC_{50} = 7.20 ± 0.16, n=4; mean ± s.e.m.). Maximal stimulation of inositol phosphates, relative to the basal level, was 1610 ± 230% (n=4). Intracellular calcium ion concentrations ($[Ca^{2+}]_i$) in the SH-SY5Y/5-HT_{2A} cell line were investigated in fura-2 loaded cells with fluorescence intensity monitored using an image analysis system. $[Ca^{2+}]_i$ was increased 4.4 fold (median value, range 1.6 - 25) in response to 5-HT (10μM) in 30/48 cells tested.

The electrophysiological response of the SH-SY5Y/5-HT_{2A} cell line to 5-HT was studied using the whole-cell patch-clamp recording technique. Patch electrodes were filled with an intracellular solution of (mM): KCl 125, $MgCl_2$ 1 HEPES 10, Na_2ATP 2, Na_2GTP 1, pH 7.4 and the extracellular medium contained (mM): NaCl 140, KCl 5, $MgCl_2$ 1, dextrose 5, HEPES 10, $CaCl_2$ 2, pH 7.4. In cells voltage-clamped at -50mV, a brief (10s) application of 5-HT (10μM) produced a slow, small inward current (median 2pA, range 1-26) in 49 out of 87 of the cells studied. In the SH-SY5Y/5-HT_{2A} cell line the inward current produced by 5-HT was abolished by prior perfusion (4 min) with the 5-HT_{2A} antagonist ketanserin (10nM; n=5) with total recovery after 10 min of perfusion with antagonist-free medium. It was evident that the current flow was increased as the holding potential was depolarized and decreased as it was hyperpolarized but we could not reverse the response. By analogy to the response in rat brain, the inward current produced by 5-HT in the SH-SY5Y/5-HT_{2A} cell line may be mediated by the closing of potassium channels. Untransfected cells did not possess specific [^{3}H]ketanserin binding, nor did they show any functional responses to 5-HT.

This cell line should therefore provide a useful model system for further studies on the human 5-HT_{2A} receptor.

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98P EVIDENCE THAT THE 5-HT_3 RECEPTOR LIGAND [^{3}H]BRL46470 LABELS ADDITIONAL 5-HT_3 RECEPTOR BINDING SITES

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The present study investigated the binding of the tritiated derivative of the atypical 5-HT_3 receptor antagonist, BRL46470 (Blackburn et al., 1993) in rat and human brain tissue, NG108-15 neuroblastoma glioma cells and human embryonic kidney cells stably transfected with the murine 5-HT_3-As receptor subunit (HEK cells; Hope et al., 1993).

Tissue preparation (NG108-15 and HEK cells were grown using standard cell culture techniques; female Wistar rat (approx. 200 g) cerebral cortex/hippocampus; human putamen (male and female patients without neurological disease, 51-83 yrs, *post-mortem* delay <30 hrs)) and radioligand binding assays were performed as described previously (Bufton et al., 1993). [^{3}H]BRL46470 (0.1-17.6 nM; 80 Ci/mmol) labelled an apparently homogenous population of specific binding sites (defined by ondansetron, 10.0 μM) in homogenates prepared from rat cortex/hippocampus, NG108-15 cells and HEK cells (Bmax = 102±16, 968±32 and 2055±105 fmol/mg protein, Kd = 1.57±0.18, 1.84±0.27 and 3.46±0.36 nM, Hill coefficients = 1.06±0.06, 0.98±0.10 and 1.02±0.08, mean±SEM, n=3, respectively) but failed to display reproducible specific binding in homogenates of human putamen (n=4). [^{3}H]Granisetron (0.1-13.8 nM; 84.5-84.8 Ci/mmol) also labelled an apparently homogenous population of specific binding sites (defined by ondansetron, 10.0 μM) in the same homogenates of rat cortex/hippocampus, NG108-15 cells and HEK cells but the densities of labelled sites were consistently lower (Bmax = 39±4, 521±47 and 870±69 fmol/mg protein, Kd = 1.55±0.61, 1.89±0.36 and 2.03±0.42 nM, Hill coefficients = 1.08±0.13, 1.00±0.09 and 0.97±0.05, mean±SEM, n=3, respectively). Furthermore, [^{3}H]granisetron also labelled an apparently homogenous population of specific binding sites in the human putamen homogenates (Bmax = 18±2 fmol/mg protein, Kd = 6.76±2.58 nM, Hill coefficient = 0.99±0.06, mean±SEM, n=4).

In competition studies, [^{3}H]BRL46470 (1 nM) binding to rat cortex/hippocampus displayed a pharmacological profile consistent with the labelling of the 5-HT_3 receptor. Thus potent 5-HT_3 receptor ligands (e.g. rank order of affinity; quipazine, (S)-zacopride, tropisetron, alosetron, meta-chlorophenylbiguanide, granisetron, (R)-zacopride, ondansetron) competed at nanomolar concentrations whilst the 5-HT_3 receptor agonists 5-HT, 2-methyl-5-HT and phenylbiguanide competed at micromolar concentrations.

Since [^{3}H]BRL46470 labelled additional specific sites compared to [^{3}H]granisetron in the HEK cell preparation (which presumably only expresses homomeric 5-HT_3 receptors comprised of the 5-HT_3-As receptor subunit) , and also the NG108-15 cell and rat cortex/hippocampus preparations, this may indicate that [^{3}H]BRL46470 labels additional sites on the 5-HT_3 receptor complex. The failure of [^{3}H]BRL46470 to label specific binding sites in the human putamen homogenate further demonstrates the distinct pharmacology of the human 5-HT_3 receptor (Bufton et al., 1993).

This work was supported by The Wellcome Trust.

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99P EFFECTS OF THE SELECTIVE 5-HT_3 RECEPTOR AGONIST 1-(*m*-CHLOROPHENYL)-BIGUANIDE ON INTRACELLULAR FREE Ca^{2+} CONCENTRATION IN UNDIFFERENTIATED N1E-115 NEUROBLASTOMA CELLS

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Application of the potent 5-HT_3 receptor agonist 1-(*m*-chlorophenyl)-biguanide (mCPBG) to N1E-115 neuroblastoma cells causes a rapid, transient inward current with a reversal potential at around 0 mV, suggesting an increased permeability to cations (Sepúlveda *et al.*, 1991). There have also been reports that 5-HT_3 receptors in N18 neuroblastoma cells display marked permeability to Ca^{2+} (Yang, 1990). Using single cell video imaging of fura-2-loaded N1E-115 cells, we aimed to determine whether activation of 5-HT_3 receptors could produce a significant increase in the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$).

mCPBG (100 - 500 nM) caused a concentration dependent increase in $[Ca^{2+}]_i$ (EC_{50} = 220 ± 3 nM; n=4). A maximal concentration of mCPBG (500 nM) caused the average cytosolic $[Ca^{2+}]_i$ to increase by 37 ± 6 nM (n=4). This response was blocked by pretreatment with ondansetron (100 nM), a selective 5-HT_3 receptor antagonist, and by removal of extracellular Ca^{2+}, the latter suggesting that the response was initiated by an influx of Ca^{2+} across the plasma membrane and not by release from internal Ca^{2+} stores. Treatment with the L-type Ca^{2+} channel activator, BAYK 8644 (1 μM), or with up to 75 mM K^+ failed to elicit an increase in $[Ca^{2+}]_i$ suggesting the absence of voltage-operated Ca^{2+} channels in these cells. Addition of mCPBG in the presence of 75 mM K^+ caused an increase in $[Ca^{2+}]_i$. Replacement of Na^+ (115 mM) with an equivalent concentration of the non-permeant cation, N-methyl-D-glucamine, resulted in a 5-fold increase in the amplitude of the response (maximum change in $[Ca^{2+}]_i$ = 180 ± 12 nM; n=4) with no significant shift in the EC_{50} for mCPBG (250 ± 10 nM).

These results demonstrate that activation of 5-HT_3 receptors in N1E-115 cells causes an increase in $[Ca^{2+}]_i$ by a direct influx of Ca^{2+} through the pore of the 5-HT_3 receptor. Depolarization of the membrane with subsequent activation of voltage-operated Ca^{2+} channels is not required for this response.

Supported by the Wellcome Trust (A.C.H. & C.W.T.). S.C.R.L. is a Royal Society University Research Fellow. Ondansetron was a gift from Glaxo Group Research.

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100P AUTORADIOGRAPHIC LOCALIZATION OF 5-HT_7 AND ITS mRNA IN RAT CNS BY RADIOLIGAND BINDING AND *IN SITU* HYBRIDIZATION HISTOCHEMISTRY

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A number of recent papers have described the cloning and expression of a novel serotonin receptor, 5-HT_7, which is positively coupled to adenylyl cyclase (Bard *et al.*, 1993; Meyerhof *et al.*, 1993; Ruat *et al.*, 1993; Shen *et al.*, 1993). We have used receptor autoradiography and *in situ* hybridization histochemistry to further delineate the distribution of the 5-HT_7 receptor and its mRNA in rat brain. Receptor autoradiographic studies were performed using 0.5 nM [^{3}H]-5-carboxamidotryptamine (5-CT) as the radioligand. Thé masks used to eliminate radioligand binding to 5-HT_{1A}, 5-$HT_{1D\alpha}$, and 5-HT_{1B} receptors were 100 nM PAPP and 160 nM (-)pindolol. *In situ* hybridization studies were carried out using antisense and sense 45mer oligonucleotide probes to the rat 5-HT_7 mRNA, which were 3'-end labelled with [^{35}S]- or [^{33}P]-dATP.

Specific binding of [^{3}H]5-CT was observed in many areas of the rat brain. Following incubation with 1 μM ergotamine, this binding was completely eliminated. Addition of PAPP and (-)pindolol to the radioligand markedly reduced the binding in a number of regions, most notably in the deep layers of the cerebral cortex, hippocampus, dorsal raphe, and spinal cord. Areas in which [^{3}H]5-CT binding remained following addition of the masks were layers 1-3 of cortex, septum, globus pallidus, thalamus, hypothalamus, centromedial amygdala, substantia nigra, periaquaductal gray, and superior colliculus. The radioligand binding in all of these areas was abolished by the addition of 5 μM methiothepin to the incubation regimen, indicating that the observed binding was attributable to 5-HT_7 receptors. The results of the *in situ* hybridization studies indicated that the distribution of the 5-HT_7 mRNA overlapped that of the 5-HT_7 receptor in some areas, and complemented it in others. The most intense hybridization signals were observed over neurons in the thalamus, the anterior hippocampal rudiment, and over CA3 in the hippocampus. Other regions containing hybridization signals included the septum, the hypothalamus, the centromedial amygdala, and the periaquaductal gray. Interestingly, two areas which exhibited a robust receptor binding signal, the globus pallidus and the substantia nigra, contained no 5-HT_7 hybridization signals, suggesting a presynaptic localization for 5-HT_7 receptors in these two related structures. The distribution of the 5-HT_7 receptor and its mRNA, described here, intimates that this novel serotonin receptor subserves functions within several brain sytems. The limbic system (centromedial amygdala, anterior hippocampal rudiment, hypothalamus) is particularly well-represented, suggesting a role for the 5-HT_7 receptor in affective processes.

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101P THE EFFECT OF 5-HT_3 RECEPTOR ANTAGONISTS ON THE DEPRESSOR RESPONSE TO COLORECTAL DISTENSION IN THE ANAESTHETISED RAT

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Distension of the GI tract evokes reflex changes in haemodynamic parameters. This is regarded as the vasomotor response to a noxious stimulation (Ness and Gebhart, 1988). 5-HT_3 receptor antagonists may attenuate this response, although certain compounds appear to be without effect in the small intestine (Moss & Sanger, 1990). We report the effects of structurally different 5-HT_3 receptor antagonists in a model of visceral nociception.

Fasted male Wistar rats were lightly anaesthetized with sodium pentobarbitone (60mgkg^{-1} i.p.) and prepared for measurement of arterial blood pressure. A latex balloon was inserted into the colorectum and inflated with saline; resulting changes in haemodynamic parameters were measured. Balloon inflation (0.5-3.0ml) elicited a volume-dependent fall in mean arterial blood pressure which allowed the construction of reproducible distension-effect curves. Morphine (5mgkg^{-1} i.v.) abolished the depressor response to colorectal distension (4.4mmHg pressor response to 1.5ml distension compared with a depressor response of 20mmHg in control animals). Naloxone (5mgkg^{-1} i.v.) reversed this effect (17mmHg depressor response at 1.5ml distension). The distension-effect curves were displaced to the right following pretreatment with ondansetron, granisetron or tropisetron. The effect of these agents on the depressor response to 1.5ml colorectal distension was compared statistically with control data (Table 1).

Table 1

		Decrease in mean arterial pressure (mmHg)			
Dose (μgkg^{-1} i.v.)	**Control**	**10**	**30**	**100**	**1000**
Ondansetron	19.7 ± 3.2	14.5 ± 5.1	-	2.0 ± 1.5*	4.0 ± 5.0*
Granisetron	28.0 ± 1.7	20.0 ± 2.5	11.5 ± 5.1*	8.2 ± 3.1*	17.5 ± 3.9*
Tropisetron	14.0 ± 1.8	-	5.8 ± 2.1*	0.4 ± 1.5*	-
n=5-7					*p<0.05

All the 5-HT_3 antagonists tested inhibited the depressor response to colorectal distension and preliminary selectivity studies showed that the hypotensive response to bolus injections of acetylcholine (0.1-100μgkg^{-1} i.v.) was not inhibited by either ondansetron or granisetron (100μgkg^{-1} i.v.) giving concentration ratios of 1.6 and 1.2 respectively.

We conclude that, in contrast with results in the small intestine, all of the 5-HT_3 antagonists tested inhibit the depressor response to a similar degree.

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102P INHIBITION OF MOTILITY INDUCED BY THE ACTIVATION OF 5-HT_1-LIKE AND 5-HT_4-LIKE RECEPTORS IN ISOLATED HUMAN COLON SMOOTH MUSCLE

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Using 5-HT indoles and substituted benzamides we previously showed that 5-HT inhibits spontaneous contractions in the circular muscle (CM) of human colon through an action at 5-HT_4 receptors (Tam et al., 1992). We have now confirmed the nature of this receptor as 5-HT_4 type using more selective 5-HT_4 agonists and antagonists; we also found that the receptor in the longitudinal muscle (LM) is 5-HT_1-like.

Activity of strips of CM or LM (taenia coli) from ascending or sigmoid colon were recorded isometrically in Krebs gassed with 95% O_2, 5% CO_2 at 37°C and 15mN tension. Results were expressed as mean±SEM (N-6-8). CM showed high levels of spontaneous contraction with low basal tone and agonist-induced inhibition of spontaneous contractions was achieved by cumulative drug addition (0.01-100μM) with a 2min contact time. Agonist effects were expressed as percentage reduction of the amplitude of spontaneous contractions measured before drug addition. LM had a high basal tone and agonist-induced relaxation of basal tone was induced with separate doses (0.01-100μM), with a 2min contact time for each dose before washout. 15min were left between doses to prevent tachyphylaxis. Antagonists were incubated for 30min before adding agonists.

In CM, 5-HT and 5-HT_4 agonist benzimidazolones (BIMU8 and BIMU1; Rizzi et al., 1992) inhibited the spontaneous contractions with EC_{50} values (95% confidence limits) of 0.13(0.04-0.42), 0.76(0.10-5.91) and 3.1(0.32-32.1)μM respectively. The selective 5-HT_4 antagonist benzimidazolone, DAU6285 (Rizzi et al., 1992), antagonised the 5-HT-induced inhibitory responses (1-10μM) yielding a pA_2 value of 6.32±0.30 (slope=0.84). The potent 5-HT_4 antagonist, GR113808 (Grossman et al., 1993), antagonised 5-HT-induced responses at 0.003μM with pK_B value of 8.89±0.24 (N=7) but higher concentrations of GR113808 (0.01-0.1μM) produced little or no further shift to the right. In 3 additional patients studied, GR113808 however, did not have any significant effect on 5-HT-induced inhibition. In LM, 5-carboxyamidotryptamine (5-CT), a potent 5-HT_1-like agonist, caused a tetrodotoxin-resistant relaxation. The maximum response was 32.7±2.17% of the relaxation induced by isoprenaline (100μM). The EC_{50} (with 95% confidence limits) for 5-CT expressed as a % of its own maximum relaxation was 0.98(0.42-2.22)μM. The effect of 5-HT was more variable and where relaxation occurred the EC_{50} was 5.73(3.48-9.01μM). The effect of 5-CT was abolished by a 5-HT_1/5-HT_2 receptor antagonist, methysergide (10μM), but was resistant to a selective 5-HT_2 receptor antagonist, ketanserin (10μM) and a 5-HT_3 receptor antagonist, ondansetron (10μM).

It is concluded that 5-HT inhibits the motility of CM via 5-HT_4-like and LM via 5-HT_1-like receptors.

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103P 5-HT_4 RECEPTOR MEDIATED CONTRACTIONS IN GUINEA-PIG DISTAL COLON CIRCULAR MUSCLE

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5-HT_4 receptors are involved in the generation of the peristaltic reflex and have been implicated in disturbances of gastrointestinal motility (Craig & Clarke, 1990). Several investigators have demonstrated 5-HT_4 receptor-mediated responses in longitudinal muscle of both proximal and distal colon of the guinea-pig; this has not previously been demonstrated in the circular muscle.

Distal colon was obtained from male Dunkin Hartley guinea-pigs (250-350g) and circular muscle strips prepared according to the method of Harry (1963). Preparations were mounted in oxygenated (95% O_2/5% CO_2) Krebs solution at 37°C containing indomethacin (3μM) to inhibit prostanoid synthesis, methiothepin (0.1μM) and granisetron (1.0μM) to pharmacologically isolate 5-HT_4 receptors. Tissues were suspended under a preload of 0.5g and responses recorded isotonically.

Non-cumulative addition of 5-HT evoked a monophasic concentration-effect curve (pEC_{50} ± s.e.m. = 7.9 ± 0.1 n=20). The potency and instrinsic activity relative to 5-HT for several other agonists was as follows (pEC_{50} ± s.e.m., [IA ± s.e.m.]); 5-MeO-T 6.9 ± 0.1 [1.0] n=8, renzapride 6.5 ± 0.2 [0.7 ± 0.1] n=6, (S)-zacopride 6.2 ± 0.4 [0.5 ± 0.1] n=3, (R)-zacopride (6.0 ± 0.1 [0.4 ± 0.1] n=3, 5-CT 5.8 ± 0.1 [0.7 ± 0.1] n=6, 2-M-5HT 4.1 ± 0.2 [0.1] n=6. Both tropisetron and SDZ 205-557 (Buchheit *et al.*, 1991) caused a concentration-dependent rightward displacement of concentration-effect curves to 5-HT yielding pA_2 estimates [slope] of 6.0 ± 0.2 [1.2] n=6 and 7.7 ± 0.5 [1.2] n=5 respectively. Cocaine (3.0μM) had no significant effect on concentration-effect curves to 5-HT, whereas exposure of tissues to pargyline (100 μM) resulted in a parallel rightward displacement (mean concentration-ratio at pEC_{50} level = 6.9 ± 2.2, n=6). In contrast pargyline evoked only a small rightward displacement of concentration-effect curves to both 5-MeO-T and renzapride (concentration-ratio ± s.e.m. = 0.7 ± 0.3 and 2.9 ± 1.3 respectively) but caused a reduction in the maximum response (>40% in both cases).

It is possible that adaptive changes, mediated by elevation of endogenous 5-HT or other monoamines (e.g. desensitisation / functional antagonism), influence responses to 5-HT receptor agonists and lead to less reliable estimates of agonist potency and intrinsic activity. Such a mechanism is less likely to influence antagonist potency and the affinities of both tropisetron and SDZ 205-557 support the presence of 5-HT_4 receptors in guinea-pig distal colon circular muscle.

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104P COMPARATIVE POTENCIES OF 5-HT_4 RECEPTOR ANTAGONISTS ON HUMAN RIGHT ATRIUM

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5-Hydroxytryptamine (5-HT) increases contractile force of human atrium through 5-HT_4 receptors, as first suggested using the 5-HT_3 receptor-selective antagonist tropisetron (ICS 205-930) which antagonised the effects of 5-HT with a pK_B of 6.7 (Kaumann *et al.*, 1990). Subsequently, more potent blockers of human atrial 5-HT_4 receptors were reported: SDZ 205-557 (pK_B=7.6) (Zerkowski *et al.*, 1993) and SB 203186 (pK_B=8.7)(Parker *et al.*, 1993). Recently, two radioligands with high affinity and selectivity for 5-HT_4 receptors have been introduced: ^{3}H-GR 113808 ($pK_D \cong$ 9.7 for rat cerebral 5-HT_4 receptors - Grossman *et al.*, 1993) and ^{125}I-SB 207710 (pK_D=10.1 for piglet cerebral 5-HT_4 receptors - Brown *et al.*, 1993). Here I report the affinity of the non-radioactive analogues of GR 113808 and SB 207710 for human atrial 5-HT_4 receptors, estimated from the antagonism of the positive inotropic effects of 5-HT. Right atrial appendages were obtained from 19 patients with coronary artery disease undergoing bypass surgery and chronically treated with β blockers. The tissues were sectioned into 2-4 strips and set up to contract at 1 Hz (37°C) in the presence of CGP20712A 300 nM (to block β_1-adrenoceptors - Kaumann, 1986), cocaine 6 μM and ascorbate 200μM as described (Kaumann *et al.*,1990). A single cumulative concentration-effect curve to 5-HT was determined per strip. One strip was used as control and 1-3 strips in the presence of antagonist (incubated 90-120 min). The experiments were concluded by the administration of (-)-isoprenaline 200 μM followed by raising the calcium concentration to 6.75 mM. Neither GR 113808 nor SB 207710 modified basal contractile force at the concentrations used. Both antagonists caused parallel shifts to the right of the concentration-effect curves for 5-HT; blockade was surmountable as judged from a lack of significant change by the antagonist in the responses to 5-HT as a function of the responses to (-)-isoprenaline and calcium 6.75 mM. Results of Table 1 are mean ± s.e.mean.

Table 1

Antagonist	N° of patients	Concentration(nM)	Slope of Schild-plot	pK_B, M
GR 113808	8	2 - 100	1.13 ± 0.12	8.79 ± 0.10
SB 207710	11	0.1-10	0.91 ± 0.10	10.14 ± 0.10

To address the question of 5-HT_4 receptor subtypes and/or species receptor homologues the potency rank order SB 207710>GR 113808≅SB 203186>SDZ 205-557>tropisetron can be compared with equivalent data obtained from brain and gut of man and other species. I am grateful to the surgeons of Papworth Hospital for the supply of atrial appendages.

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105P TISSUE AND AGONIST DEPENDENT NON-SURMOUNTABLE ANTAGONISM BY THE SELECTIVE 5-HT_4 RECEPTOR ANTAGONIST SB 204070

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In the present study we have evaluated the non-surmountable actions of the potent and selective 5-HT_4 receptor antagonist SB 204070 (Wardle *et al.*, 1993) at 5-HT_4 receptors in guinea-pig distal colon (GPDC) and rat oesophagus (RO). Both preparations were set up as described previously (Baxter *et al.*, 1992). Up to three agonist concentration-effect curves were constructed in each preparation and the effects of SB 204070 were evaluated after an equilibration period of at least one hour.

At low concentrations (0.01-0.03 nM), SB 204070 was a surmountable antagonist of 5-HT in GPDC yielding an apparent pA_2 estimate of approximately 10.8. Higher concentrations produced a reversible depression of the maximum response (Wardle *et al.*, 1993). We now show that *all* effective concentrations of SB 204070 caused non-surmountable antagonism of responses to the partial agonist BIMU1 (Rizzi *et al.*, 1992) in GPDC. Likewise, all effective concentrations of SB 204070 caused non-surmountable antagonism of 5-HT in RO. The effects of SB 204070 in RO were not significantly different following one or two hour incubation and were reversed upon washing. SB 204070 did not affect contractile responses to carbachol in either RO or GPDC at concentrations up to 1.0 μM.

SB 204070 (nM)	GPDC: 5-HT		GPDC: BIMU 1		RO: 5-HT	
	pEC_{50}	α	pEC_{50}	α	pEC_{50}	α
Control	9.0 ± 0.2	1.0 ± 0	7.0 ± 0.1	0.6 ± 0.05	7.8± 0.1	1.0 ± 0.02
SB 204070 (0.01)	8.7 ± 0.1	1.0 ± 0.02	6.7± 0.1	0.5 ± 0.04	7.9 ± 0.1	1.1 ± 0.03
SB 204070 (0.03)	8.5 ± 0.1	1.0 ± 0.05	6.3 ± 0.2	0.4 ± 0.10	7.6 ± 0.2	0.9 ± 0.10
SB 204070 (0.1)	8.2 ± 0.1	0.9 ± 0.03	6.1 ± 0.2	0.2 ± 0.02	7.5 ± 0.2	0.6 ± 0.10
SB 204070 (0.3)	7.6 ± 0.1	0.8 ± 0.01		0.0 ± 0.00	7.3 ± 0.2	0.4 ± 0.12
SB 204070 (1.0)	7.2 ± 0.1	0.5 ± 0.02	-	-	-	0.0 ± 0.00
SB 204070 (3.0)	6.5 ± 0.1	0.2 ± 0.04	-	-	-	-

All data are mean ± s.e.m. α= maximum response relative to 5-HT control maximum. (n = 4 - 6)

Although there are many plausible mechanisms for non-surmountable antagonism, the most likely explanation for the present results is that SB 204070 acts as a 'pseudo irreversible antagonist'. The tissue and agonist dependent differences may be explained by the presence of high and low 'effective receptor reserve' in GPDC and RO respectively. If this is the case, the 5-HT_4 receptor mediating the contractile response of GPDC may be unique amongst 5-HT receptors in possessing a substantial receptor reserve for 5-HT.

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106P (+) WAY 100135 INHIBITS ANTINATRIURESIS FOLLOWING HIGH SODIUM INTAKE IN CONDITIONS OF SELECTIVE TYPE A MONOAMINE OXIDASE INHIBITION

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Dopamine (DA) and 5-hydroxytryptamine (5-HT) have been found, respectively, to decrease and increase the tubular reabsorption of Na^+. In kidney, the two amines share common synthetic and metabolic pathways; their respective amino acid precursors undergo decarboxylation by tubular aromatic L-amino acid decarboxylase whereas type A monoamine oxidase (MAO-A), the predominant form of MAO in rat renal tissues, converts DA into 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-HT into 5-hydroxyindolacetic acid (5-HIAA). More recently, in conditions of high sodium intake we have found that selective inhibition of MAO-A results in a marked antinatriuretic effect accompanied by an increase in the urinary excretion of 5-HT. In the human and rat kidney, 5-HT_{1A} receptors have been found to be specifically localized in tubular epithelial cells of nephron segments particularly involved in the regulation of salt and water transport (Raymond et al., 1993). The present study has examined the effect of (+)-WAY 100135, a selective antagonist of 5-HT_{1A} receptors (Routledge et al., 1992), and ketanserin, an antagonist of 5-HT_2 receptors, on the urinary excretion of Na^+, K^+, DA, 5-HT and their metabolites in rats treated with the selective MAO-A inhibitor Ro 41-1049 (50 μmol kg^{-1} day^{-1}) in conditions of high salt diet (HSD; 1.0% NaCl in drinking water). Male Wistar rats were placed in metabolic cages and 24 hours acidified urine samples were collected for the quantification of DA, DOPAC, homovanillic acid (HVA), 5-HT and 5-HIAA by means of HPLC-EC and of Na^+ and K^+ by flame photometry. Animals were given tap water (normal salt diet) in the first 7 days of the study and then challenged to a HSD for another 7 days; Ro 41-1049 was given in drinking water only in the last 3 days of HSD, whereas (+)-WAY 100135 (10 mg kg^{-1} day^{-1}, s.c) or ketanserin (2 mg kg^{-1} day^{-1}, s.c.) were administered in the last 4 days of the HSD period. Daily liquid intake (in ml kg^{-1} day^{-1}) during HSD (303±27) was found to be higher ($P<0.02$) than that during the NSD period (171±5). Daily urinary excretion (in nmol kg^{-1} day^{-1}) of DA (94±3), DOPAC (284±28), HVA (2117±379), 5-HT (387±37) and 5-HIAA (1237±39) in the HSD period was similar or higher than that in the NSD period (DA=91±5, DOPAC=270±24, HVA=1001±145, 5-HT=265±20, 5-HIAA=736±37). The administration of Ro 41-1049 on 3 consecutive days was found to reduce the urinary excretion of DA, DOPAC and HVA, respectively, by 27-34%, 33-70% and 19-83%; the urinary excretion of 5-HT increased 2-fold and the levels of 5-HIAA were reduced by 18-58%. During HSD, daily urinary excretion of Na^+ increased 7-fold (from 7.6±0.4 to 54.2±3.0 mmol kg^{-1} day^{-1}), without changes in the urinary excretion of K^+ (from 12.3±0.4 to 13.2±0.3 mmol kg^{-1} day^{-1}). MAO-A inhibition during HSD was found to produce a 37-71% decrease in Na^+ excretion and urine volume without changes in K^+ excretion. Administration of (+) WAY 100135, but not of ketanserin, was found to inhibit the antinatriuretic effect induced by Ro 41-1049 during HSD. It is suggested that MAO-A inhibition during high sodium intake leads to an increased availability of 5-HT in renal tissues, the effect of which is a decrease in the urinary excretion of Na^+, involving the activation of tubular 5-HT_{1A} receptors.

This study was supported by grant number PBIC/C/CEN 1139/92 from the JNICT.

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107P 5-HT_1-LIKE RECEPTORS AND A NOVEL RECEPTOR MEDIATE 5-HT-INDUCED DECREASES OF THE CANINE EXTERNAL CAROTID BLOOD FLOW

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(Introduced by P.R. Saxena)

Intracarotid (i.c.) infusion of 5-hydroxytryptamine (5-HT) increases external carotid blood flow (ECBF) in intact dogs via 5-HT_1-like receptors (Villalón et al., 1993); in contrast, 5-HT decreases ECBF in vagosympathectomized dogs, but the mechanisms and/or receptors involved in this last effect have not been completely characterized (Saxena, 1972). This study has investigated the receptor(s) involved in 5-HT-induced decrease in ECBF in 22 vagosympathectomized dogs under anaesthesia with pentobarbital (30 mg kg^{-1}, i.v.). The baseline values of heart rate and mean arterial blood pressure were 169±5 beats min^{-1} and 119±5 mmHg, respectively. I.c. infusion of 5-HT (3, 10, 30 and 100 $\mu g\ min^{-1}$) elicited dose-dependent decreases in ECBF (Table 1) without changes in mean arterial blood pressure or heart rate.

Table 1: Effect of various antagonists on decreases in ECBF (ml min^{-1}) elicited by 5-HT ($\mu g\ min^{-1}$) in vagosympathectomized dogs.

Antagonist	Dose	n	3		10		30		100	
	mg kg^{-1}		Before	After	Before	After	Before	After	Before	After
Phentolamine	1.0	3	-33±2	-31±7	-50±10	-49±13	-90±37	-75±31	-126±70	-82±35
Propranolol	1.0	3	-38±11	-38±14	-55±14	-53±17	-65±12	-58±15	-64±13	-59±16
Fluoxetine	1.0	3	-41±19	-62±16	-66±23	-76±25	-90±25	-93±30	-103±41	-99±35
Methiothepin[a]	3.0	5	-29±8	-4±1*	-42±10	-13±2*	-45±10	-30±5	-46±10	-48±11
Ritanserin	0.3	3	-25±11	-34±9	-42±13	-57±9	-52±11	-67±8	-58±12	-73±10
MDL 72222	0.3	3	-37±4	-50±7	-56±6	-70±18	-67±3	-79±18	-78±3	-83±17
ICS 205-930	3.0	3	-53±13	-49±11	-69±15	-71±17	-81±20	-90±23	-87±24	-98±35

a, Dogs were pretreated with chlorisondamine (5 mg kg^{-1}, i.v.) to avoid methiothepin-induced hypotension; *, ($P<0.05$) from the corresponding response before injection of the antagonist. More than one antagonist (except methiothepin) was used in an animal.

Apart from the blockade of the response to 3 and 10 $\mu g\ min^{-1}$ of 5-HT by methiothepin, none of the drugs employed, which included blockers of adrenoceptors (α and ß), 5-HT-uptake and the four 5-HT receptors, antagonized the decreases in ECBF (Table 1) or conductance by 5-HT. In addition, the responses to 5-HT remained unchanged after brompheniramine, cimetidine, haloperidol, atropine and verapamil, and methiothepin (3 mg kg^{-1}) completely blocked sumatriptan-induced decreases in ECBF and conductance (data not shown). It is therefore suggested that 5-HT decreases the ECBF and conductance in vagosympathectomized dogs by activation of 5-HT_1-like receptors; interestingly, at higher doses of 5-HT, a new 5-HT receptor type, which is apparently different from the four types (5-HT_1, 5-HT_2, 5-HT_3 or 5-HT_4) characterized thus far, seems to be involved.

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108P THE EFFECT OF ASPIRIN (40 mg/DAY) ON PLATELET-INDUCED CONTRACTIONS OF THE HUMAN ISOLATED CORONARY ARTERY: A ROLE FOR POTENTIAL THERAPEUTIC 5-HT RECEPTOR ANTAGONISTS?

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The beneficial effect of low dose aspirin in the prevention of coronary vasospasm is well documented (The RISC Group, 1990). In the present study we investigated the contractile effect of human washed platelets on the human isolated coronary artery. We concentrated on the effect of low dose aspirin (40 mg per day), taken by the platelet-donor, and on the efficacy of thromboxane (Tx)A_2- and 5-HT_2 receptor antagonists. Human coronary artery segments (endothelium intact), obtained from 10 heart beating organ donors (heart valves used for transplantation purposes; 8 male, 2 female; age: 7-48 years) were suspended in an organ bath set-up (8 ml) for isometric tension measurement. Data are expressed as mean ± s.e.mean.
Platelets (10^9-3.10^{10}/l) elicited concentration dependent contractile responses of the coronary artery segments (n=6) reaching 28.4 ± 7.1 % of contractions induced by 100 mM K^+. The contraction to platelets was increased non-significantly after pretreatment of the vessel segment with L-NAME (30 μM), reaching 40.2 ± 19.2 of contraction to 100 mM K^+. Contraction was significantly attenuated after pretreatment of the vessel segments with ketanserin (5-HT_2 receptor antagonist, 1 μM) or SQ30741 (TxA_2 receptor antagonist (Schumacher et al., 1989); 0.01 μM), reaching 8.8 ± 2.3 % and 3.2 ± 2.2 % of contraction to 100 mM K^+, respectively. Platelets obtained from the same platelet-donors after taking aspirin (40 mg/day for 7-13 days) caused significantly lower contractile responses (7.6 ± 2.7 % of 100 mM K^+) associated with an almost selective inhibition of the synthesis of thromboxane measured in the organ bath solution (TxB_2, Radio Immuno Assay (RIA), untreated platelets: 2.19 ± 0.43 nM; aspirin-treated platelets: 0.66 ± 0.05 nM). The amount of PGE_2 and 6-keto-$PGF_{1\alpha}$, measured (RIA) in the organ bath solution, was not significantly altered after treatment of the platelet donors with aspirin (untreated platelets: 0.42 ± 0.16 nM and 0.29 ± 0.08 nM, respectively; aspirin-treated platelets: 0.33 ± 0.17 nM and 0.54 ± 0.26 nM, respectively). The amount of 5-HT secreted in the organ bath also remained unchanged (HPLC, untreated platelets: 65.17 ± 9.94 nM; aspirin-treated platelets: 64.03 ± 8.98 nM). This explains why ketanserin significantly attenuated the residual contractile responses, caused by platelets obtained from aspirin-treated subjects (to 0.9 ± 1.9 % of 100 mM K^+), whereas SQ30741 caused minor, non-significant additional attenuation (to 4.0 ± 1.8 % of 100 mM K^+). The results of the present study suggest that additional antagonism of the contractile 5-HT receptors in the coronary artery may increase the efficacy of low dose aspirin in-vivo.

Study supported by *The Netherlands Heart Foundation*, grant 89.252.

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109P PLASMA GLUCOSE AND CARDIOVASCULAR STUDIES IN THE RAT WITH WAY100635, A SELECTIVE, POTENT 5-HT_{1A} RECEPTOR ANTAGONIST

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8-OH-DPAT decreases mean arterial blood pressure (MABP) and heart rate (HR) and increases plasma glucose levels (PGL) in the rat via an agonist action on central, post-synaptic 5-HT_{1A} receptors (Chaouloff *et al.*, 1990; Fozard *et al.*, 1987). We have examined WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide, trihydrochloride, hydrate-W635) a potent and selective 5-HT_{1A} receptor antagonist (Fletcher *et al.*, 1994) both for intrinsic effects on, and as an antagonist of 8-OH-DPAT-induced changes in MABP, HR and PGL.

Male Sprague-Dawley rats (330-520 g) were prepared under halothane anaesthesia, with carotid and jugular vein cannulae, fasted overnight, then placed in semi-restraint tubes . 2 pre-injection readings of MABP and HR and arterial blood samples (0.3 ml) were taken at t=-25 and -20 min. W635 (10, 30 or 100 µg/kg i.v.) or saline vehicle (1 ml/kg i.v.) were injected at t=-15 min. Immediately prior to injection with 8-OH-DPAT (100 µg/kg i.v.) or saline vehicle at t=0, readings of MABP, HR and PGL were made. Further measurements were then made at t=10, 20 and 30 min. Plasma samples were assayed for glucose in triplicate using a Beckman Glucose Analyser 2. Data were analysed by 2 factor ANOVA (n=6 per treatment group).

Baseline levels of MABP, HR and PGL were 143.4±1.5 mmHg, 439.7±4.9 b/min and 110.8±2.0 mg/dl respectively. W635 did not affect any of the parameters except for an increase in HR at the highest dose. 8-OH-DPAT significantly decreased MABP and HR and elevated PGL (Table 1). W635 dose-dependently antagonised all of the 8-OH-DPAT-induced responses, with almost complete blockade being observed at a dose of 100 µg/kg (Table 1).

TABLE 1 Maximum changes induced by 8-OH-DPAT following W635 pretreatment compared to vehicle/8-OH-DPAT (*p<0.05) treated rats at the equivalent time point.

	Vehicle/8-OH-DPAT	W635(10µg/kg)/8-OH-DPAT	W635(30µg/kg)/8-OH-DPAT	W635(100µg/kg)/8-OH-DPAT
MABP (mmHg)	-44.0±3.6	-28.6±6.2 *	-11.5±3.1 *	-9.7±3.8 *
HR (b/min)	-116.8±14.2	-72.8±17.0 *	-27.8±20.3 *	-15.8±8.8 *
PGL (mg/dl)	28.5±7.0	10.8±6.2 *	6.6±5.2 *	1.2±4.6 *

In conclusion these data demonstrate that W635 is a potent 5-HT_{1A} receptor antagonist which inhibits the hypotension, bradycardia and hyperglycaemia induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT.

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110P 5-HYDROXYTRYPTAMINE 5-HT_{1B} RECEPTORS MEDIATE INHIBITION OF ADENYLYL CYCLASE IN A RAT RENAL MESANGIAL CELL LINE

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5-Hydroxytryptamine (5-HT) 5-HT_{1B} receptors have been shown to function as presynaptic receptors in rat brain (Engel et al., 1986). In addition, there is evidence for 5-HT_{1B} receptors being present in the periphery, where they mediate inhibition of noradrenaline release (rat vena cava; Molderings et al., 1987), inhibition of adenylyl cyclase (opossum kidney epithelial OK cell line; Murphy & Bylund, 1989), cell growth (Chinese hamster lung fibroblasts; Seuwen et al., 1988) and vascular contraction (rat tail artery; Craig & Martin, 1993). We now show that 5-HT_{1B} receptors mediate inhibition of adenylyl cyclase activity in a rat renal mesangial cell line.

A clonal cell line derived from rat renal mesangial cells was grown in Dulbecco's modified Eagle medium containing 10 % foetal calf serum, 100 IU/ml penicillin and 100 µg/ml streptomycin. The cells had all the characteristics of mesangial cells, according to various morphological, biochemical and functional criteria (Pfeilschifter & Merriweather, 1993). They were used at confluency in 24-well plates for measurement of [^{3}H]adenine conversion to [^{3}H]cyclic AMP as previously described (Murphy & Bylund, 1989). Mean values ± s.e.mean were calculated from 3 individual experiments.

5-HT and 5-HT_1 receptor agonists induced a concentration-dependent inhibition of forskolin (10 µM)- stimulated adenylyl cyclase activity in rat mesangial cells, reaching 60-70 % maximal inhibition. The rank order of potency was (pEC_{50}): ergotamine (9.58 ± 0.51) > RU 24969 (8.67 ± 0.23) > 5-CT (8.42 ± 0.06) > CP 93129 (8.15 ± 0.27) > 5-HT (7.75 ± 0.11) > sumatriptan (6.29 ± 0.30) > 8-OH-DPAT (4.32 ± 0.15). The 5-HT_2 receptor agonist, DOI, and the 5-HT_4 receptor agonist, renzapride, were devoid of effect, up to 0.1 mM. The putative antagonists, (-)cyanopindolol and (-)propranolol induced weak inhibition (maximum: 25-30 %), whereas methiothepin and rauwolscine had no effects per se (up to 10 µM). Methiothepin (0.1, 1 and 10 µM) produced concentration-dependent shifts of the 5-HT curve to the right, yielding a pA_2 value of 8.36 (Schild slope 0.87). (-)Propranolol (1 µM) also shifted the 5-HT curve to the right (pK_B 7.01 ± 0.22), whereas rauwolscine (10 µM) did not.

The above pharmacological profile is typical for 5-HT_{1B} receptors which are the rat homologues of human 5-$HT_{1D\beta}$ receptors; it is possible, therefore, that human mesangial cells will express the latter negatively coupled to adenylyl cyclase. By analogy with other cells and tissues expressing 5-HT_{1B} receptors, a role in cell contraction -and thereby regulation of glomerular filtration rate- or in cell proliferation might be expected.

5-CT: 5-carboxamidotryptamine; RU 24969: 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridyl)-1H indole; CP 93129: 3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolo[3,2-b]pyrid-5-one; 8-OH-DPAT: 8-hydroxy-2-(N,N-dipropylamino)tetralin; DOI: 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane.

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111P METHIOTHEPIN ANTAGONISM DOES NOT ATTENUATE THE BRADYCARDIA ASSOCIATED WITH SEVERE HAEMORRHAGE IN THE ANAESTHETISED RAT

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The cardiovascular response to blood loss is biphasic in both man (e.g. Barcroft *et al.*, 1944) and animals (e.g. Little *et al.*, 1989). The first phase comprises a tachycardia while arterial blood pressure is maintained by the baroreflex, followed by a second phase comprising a reflex bradycardia and fall in blood pressure. It has been suggested that this second "depressor" reflex is due to the activation of cardiac-vagal afferent C-fibres (Öberg and Thorén, 1972) which forms part of the "Bezold-Jarisch" response. However, the precise nature of the "depressor" reflex has recently been questioned (Evans *et al.*, 1993). Recent studies (Bogle *et al.*, 1990) have shown that the "Bezold-Jarisch" reflex can be blocked by the central administration of the non-specific 5HT receptor antagonist methiothepin. The aim of the present study was to determine the effects of methiothepin on the cardiovascular response to blood loss.

Experiments were performed on 3 groups of 6 male Wistar rats (243-251g). Stainless guide cannulae were implanted into the fourth cerebral ventricle (ic) under pentobarbitone anaesthesia (80mg.kg^{-1} ip) 4-5 days prior to the study. On the day of the study anaesthesia was induced with isoflurane (3.5% in O_2) and maintained with alphadolone/alphaxolone (18-24 mg.kg^{-1}.h^{-1} iv). The electrocardiograph was recorded via needle electrodes attached to the skin of the ventrum, blood pressure via the ventral tail artery and body temperature via a rectal probe. Body temperature was maintained constant at 38.1±0.1°C (mean ± s.e.mean) using external heating.

Groups I and II received a haemorrhage of 40% total blood volume (BV; estimated as 6.13 ml.100g^{-1} body weight) at 2% BV.min^{-1}. Group I received vehicle (0.9% saline, 5µl ic) 5 min prior to the haemorrhage. Group II received methiothepin (200 µg.kg^{-1} in 5µl) via the same route, and Group III received methiothepin but no haemorrhage. Animals in Group I displayed an initial tachycardia followed by bradycardia (heart period, HP, decreasing significantly [$P<0.05$ paired t-test] by 8±2 ms after 6.6±0.0% BV loss from a control value of 144±3ms, followed by a significant increase of 36±6 ms after a 26.5±0.1% BV loss). Group II animals displayed an exaggerated bradycardic response, with HP increasing by 62±7 ms, which was significantly greater than that seen in Group I, after a significantly smaller blood loss (23.2±0.2% BV, unpaired t-test). Methiothepin alone (Group III) had no significant effect on heart period.

These results indicate that the non-specific 5HT receptor antagonist methiothepin is unable to attenuate the reflex bradycardia which accompanies severe haemorrhage, unlike its actions on the "Bezold-Jarisch" response, and suggests that the two responses may be mediated by different central nervous pathways.

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112P ACTIVATION OF PROTEIN KINASE C POTENTIATES AGONIST-INDUCED STIMULATION OF cAMP PRODUCTION IN CULTURED RAT RETINAL PIGMENT EPITHELIAL CELLS

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Crosstalk between cell-surface receptors coupled to second messengers provides the cell with an extended ability to modulate intracellular signals. The concerted action of drugs enables a precise fine-tuning of the functional aspects of a cell. We have previously demonstrated that cultured rat retinal pigment epithelial (RPE) cells possess a 5-HT_{2A} receptor coupled to mobilisation of both inositol phosphates production and intracellular calcium (Osborne *et al*, 1993). Here we show that these receptors are indirectly linked to the stimulation of adenylate cyclase.

Cyclic AMP levels were measured by the competition between a standard amount of tritiated cAMP and cellular cAMP for sites on a binding protein prepared from bovine adrenal cortex. A 10 minute incubation with serotonin (100µM) alone has no effect on basal levels of cAMP, however a 5 minute preincubation potentiates the forskolin (5µM), isoproterenol (1µM) and NECA (10µM) induced stimulation of cAMP production over 5 minutes by 233 ± 13.8%, 396.8 ± 40.0% and 751.9 ± 140.8% respectively. The EC_{50} values for the action of serotonin on forskolin and NECA was 0.4µM and 0.2µM respectively. this is similar to the EC_{50} value for the stimulation of inositol phosphates production via the 5-HT_{2A} receptor, found to be 0.9µM (Osborne *et al*, 1993). The serotonin effect on the agonist-induced stimulation of cAMP was attenuated by the 5-HT_2-antagonist ketanserin further suggesting the direct involvement of the 5-HT_{2A} receptors. Initial studies show that serotonin activates protein kinase C (PKC) in cultured RPE cells; addition therefore of the PKC activator, phorbol dibutyrate, mimicked the serotonin effect, while the inactive, non-tumour promoting 4α-phorbol dibutyrate had no influence. The action of serotonin and phorbol dibutyrate was inhibited by the putative PKC inhibitor, staurosporin. These data thus point to the involvement of PKC in the potentiating action of serotonin. Bradykinin, which also stimulates inositol phosphates production, mimicked the effect of serotonin on isoproterenol and forskolin stimulation of cAMP levels with EC_{50} values of 0.02µM and 0.07µM respectively.

The present data show that receptors linked to the stimulation of inositol phosphates can potentiate receptor-mediated activation of cAMP. This process involves the enzyme protein kinase C. The physiological importance of this crosstalk between receptors involving different transduction processes on the RPE cells has still to be assessed.

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113P THE ROLE OF DIFFERENT CALCIUM COMPARTMENTS IN THE MUSCARINIC AND HIGH POTASSIUM-EVOKED RELEASE OF 5-HT FROM ISOLATED RABBIT ILEUM

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5-HT is accumulated in the enterochromaffin cells (ECs) of the intestinal mucosa and can be released by cholinergic stimuli as well as by high-potassium (Racké & Schwörer, 1991). The present experiments should elucidate the role of extra- and intracellular calcium in the stimulus secretion coupling of 5-HT release from ECs. Rabbits were killed by a sharp blow to the back of the neck and exsanguinated and a perfusion of the small intestine via the upper mesenteric artery with oxygenated and warmed (37°C) Krebs-HEPES solution was performed for 20 min. This treatment has been found to enhance the responsiveness to different stimuli (Reimann & Hering, 1992). Thereupon, segments of the ileum were prepared and fixed in modified Ussing-chambers. The incubation medium (Krebs-HEPES solution containing 1,2 µM calcium) was changed every 10 min at both sides. 5-HT in the serosal compartment was determined by HPLC with electrochemical detection. Calcium antagonists were present from the onset of incubation, oxotremorine or high-potassium were added from the 50th min to the end of the experiments.

The basal outflow of 5-HT to the serosal side amounted to 1.4 ± 0.16 pmol/cm^2 /10min, n=36 (means ± s.e.m.) and was not significantly reduced when calcium was omitted from 20 min of incubation onwards. Similarly neither the dihydropyridine calcium channel blocker nifedipine nor ω-conotoxin had any effect on basal outflow while 1 mM gadolinium chloride reduced basal release by 50%. High-potassium (45 mM) increased 5-HT maximal overflow to 11.0 ± 1.74 pmol/cm^2/10min, n=15, this effect was markedly reduced (1.6 ± 0.19 pmol/cm^2/ 10min, n=4) under calcium-free conditions. The maximal outflow evoked by 10 µM oxotremorine (18.6 ± 2,15 pmol/cm^2/10 min, n=19) was not significantly diminished after omission of calcium although delayed in onset. Similarly, nifedipine (1 and 10 µM) reduced the high-potassium evoked release up to 76 ± 4% (n=4) without affecting the oxotremorine stimulus. Also 1 mM gadolinium chloride abolished the response to high-potassium while the muscarinic effect was only reduced by 50%. ω-Conotoxin had no inhibitory effect on either stimulus, but enhanced the high-potassium evoked release without affecting the inhibitory effect of nifedipine. Ryanodine (10 to 100 µM) markedly reduced the oxotremorine response to 7.4 ± 1.0 pmol/cm^2/10min, n=8.

It is concluded that 5-HT release from ECs evoked by high-potassium is triggered by calcium influx through voltage-sensitive calcium channels, which are dihydropyridine-sensitive. In contrast, muscarinic evoked 5-HT release causes the mobilization of intracellular calcium stores, which are in part ryanodine sensitive.

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114P EFFECTS OF PHOSPHORAMIDON AND PEPSTATIN A ON THE SECRETION OF ENDOTHELIN-1 AND BIG ENDOTHELIN-1 IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS: MEASUREMENT BY TWO-SITE ELISAs

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Endothelin-1 (ET-1) is synthesised within endothelial cells from its precursor, big ET-1 by one or more unidentified endothelin converting enzymes (ECE). Big ET-1 has little vasoconstrictor activity in isolated human vessels (Howard, *et al.*,1992) suggesting that inhibitors of ECE may be of potential therapeutic value in clinical conditions where ET-1 levels are elevated. In animals, the effects of phosphoramidon, have been extensively studied both *in vitro* and *in vivo* (Opgenorth, *et al.*, 1992). Human cathepsin E, an aspartyl protease, also synthesises biologically active ET-1 from big ET-1 *in vitro* without any further degradation (Lees, *et al.*,1990). Our aim was to use sensitive and highly specific two-site enzyme-linked immunosorbent assays (ELISA) and cultured human umbilical vein endothelial cells (HUVEC), to compare the effects of these two potential inhibitors of ECE on the synthesis of ET-1.

HUVEC were prepared by collagenase dispersion and first passage confluent monolayers were incubated for 20 h with either phosphoramidon or pepstatin A (an aspartyl protease inhibitor) at 10^{-4}M. ET-1 and big ET-1 secreted into the media were measured in parallel using two-site ELISAs (Amersham) over the subsequent 12 hours. Neither treatment reduced cell viability as determined by trypan blue exclusion. Standards and samples were incubated in duplicate in 96-well plates precoated with anti-ET-1$_{(15\text{-}21)}$ or anti-big ET-1$_{(22\text{-}38)}$ and unbound material was removed by washing. Bound ET-1 or big ET-1 was detected using anti-ET-1 horseradish peroxidase conjugate followed by a chromogenic substrate solution. The absorbance at 450nm of each well was measured using a 96-well spectrophotometer and ET-1 or big ET-1 concentrations were determined by interpolation from a standard curve.

The sensitivities of detection, intra- and inter-assay coefficients of variation were 0.5 fmol/well, 3.5% and 16.8% for the ET-1 ELISA and 0.31 fmol/well, 3.6% and 9% for the big ET-1 ELISA, respectively. The ET-1 assay cross-reacted with ET-2 >100% and with all other ET family peptides or fragments and unrelated peptides <0.07%. The big ET-1 assay cross-reacted with ET-1 <0.03% and <0.006% with all other peptides tested. Dilution curves of conditioned media from five cultures of HUVEC were parallel to those of the standard peptides indicating that the assays detected the expected authentic peptides. This together with the high specificity of the ELISAs allowed the direct quantitation of ET-1 and big ET-1 without extraction or concentration.

Measurements were made from two wells of three separate cultures of HUVEC and the amount of peptides compared using analysis of variance (P<0.01) The concentrations of ET-1 and big ET-1 secreted into the media showed a linear increase over a time course of 12 hours. Phosphoramidon reduced ET-1 secreted into the medium to 48% with a corresponding increase in big ET-1 to 231% of control levels. Pepstatin A also reduced ET-1 levels to 62% without any significant change to big ET-1 secretion from controls. These data suggest that in HUVEC, in addition to a phosphoramidon-sensitive ECE, an aspartyl protease (possibly cathepsin E) may also play a role in the synthesis of ET-1.

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115P EFFECTS OF Ro 46-2005, AN ANTAGONIST OF ET_A AND ET_B RECEPTORS, ON THE RESPONSES TO ENDOTHELIN-1 *IN VITRO* AND *IN VIVO*

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We have previously examined the effects of the selective ET_A receptor antagonists, BQ 123 and FR 139317 against agonist responses in the rat isolated aorta (RA) and trachea (RT) and concluded that endothelin-1 (ET-1) induced contraction is mediated by stimulation of ET_A receptors in the RA and ET_B receptors in the RT (Kengatharan et al., 1993). Ro 46-2005 (4-t-butyl-N[6(2-hydroxyethoxy)-5-(3-methoxyphenoxy)-4-pyrimidinyl(benzenesulphonamide]) is a non-selective ET_A/ET_B receptor antagonist based on its equipotent activity in RA against ET-1 induced contraction and against sarafotoxin 6c (STX6c; a selective ET_B agonist) induced relaxation in the rat mesenteric artery (Clozel et al., 1993). In this study, we have used Ro 46-2005 to characterise further the endothelin receptors in RA and RT and we have also examined its effect on blood pressure responses to ET-1 in pithed rats.

The experimental protocol used in the *in vitro* (RA and RT) experiments to determine antagonist potency (pA_2 or pK_B values) was described previously (Kengatharan et al., 1993). In the *in vivo* experiments, male Sprague Dawley rats (~300g) were anaesthetised with halothane (4% v/v in O_2), pithed and artificially ventilated (room air at 95 breaths per min). The changes in diastolic blood pressue (DBP) to ET-1 (0.5 nmol/kg; i.v.) were assessed 5 min after adminstration of vehicle (saline 1ml/kg; i.v.), BQ 123 or FR 139317 (both 1mg/kg, i.v.). Ro 46-2005 (10mg/kg, i.v.) was given 10 min before challenging with ET-1. The values are expressed as mean ± s.e. mean from groups of 4-11 animals. Statistical significance was tested using either ANOVA (Bonferroni method) or Student's t-test where appropriate with $p<0.05$ considered significant.

In the RA, Ro 46-2005 was a competitive antagonist against ET-1 (pA_2 = 5.70±0.13, Schild plot slope = 1.03±0.17; n=4). However, in RT, Ro 46-2005 (100μM) did not affect the responses to ET-1 (control EC_{50} = 30.2±6.1 nM compared with 23.4±4.4 nM; n=7) but it antagonised STX6c (pK_B = 4.96±0.21; n=5). BQ 123 (pA_2 = 6.80) and FR 139317 (pA_2 = 6.74) were competitive antagonists in the RA against ET-1, but were ineffective in RT against ET-1 and STX6c (Kengatharan et al., 1993). Progressive, significant rightward shifts of ET-1 response curves in RT occurred after Ro 46-2005 (10, 30 and 100μM) given together with BQ 123 (10μM). In contrast however, combination of FR 139317 (10μM) with Ro 46-2005 (100μM) was ineffective against ET-1 in this tissue (control EC_{50} = 18.3±2.7 nM compared with 16.0±2.2 nM; n=6).

The basal DBP in pithed control rats was 45.7±2.4 mmHg (n=11). In these animals, ET-1 caused a transient depressor response (-8.9±1.3 mmHg) followed by a rise in DBP (+24.4±2.2 mmHg). Neither BQ 123 nor FR 139317 affected the fall in DBP to ET-1 (-9.9±1.0 mmHg, n=5 and -8.7±1.2 mmHg, n=9, respectively; $p>0.05$). In contrast, Ro 46-2005 antagonised significantly the fall in DBP (-0.8±0.6 mmHg; n=5; $p<0.001$). The pressor response to ET-1 was antagonised by FR 139317 (+1.4±0.6 mmHg, $p<0.001$) and BQ 123 (+2.3±0.7 mmHg) but it was accentuated by Ro 46-2005 (+59.2±5.5 mmHg, $p<0.001$).

In summary, Ro 46-2005 antagonised competively ET-1 responses in RA confirming that this compound possesses ET_A receptor blocking properties. In RT, Ro 46-2005 antagonised the ET_B selective agonist STX6c but only antagonised the effects of ET-1 when given together with BQ 123. These results suggest the presence of both ET_A and ET_B receptors in this tissue as proposed by Henry (1993). The fact that FR 139317 did not behave like BQ 123 in combination with Ro 46-2005 in the RT cannot be explained at present but may reflect difference between the ET_A receptors present in RT and RA. Ro 46-2005 (10mg/kg, iv.) appeared to demonstrate predominantly ET_B antagonist properties in pithed rats evidenced by inhibition of the ET-1 induced depressor effect and potentiation of the pressor response.

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116P THE EFFECTS OF TWO ET_A/ET_B RECEPTOR ANTAGONISTS, PD 145065 AND Ro 46-2005, ON CONTRACTIONS INDUCED BY ET-1 OR IRL 1620 IN THE ISOLATED BRONCHUS OF THE GUINEA-PIG

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In the isolated upper bronchus of the guinea-pig, ET-1, ET-3, IRL 1620 and SX6c were found to be equipotent (Battistini et al., 1993; Hay, 1992) and the contractions were inhibited by PD 145065 (Battistini et al., 1993). Thus, endothelins produce bronchoconstrictions which are mediated by ET_B receptors (Battistini et al., 1993). Here, we have examined the effectiveness of Ro 46-2005 (Clozel et al., 1993) and PD 145065 (Cody et al., 1993), two structurally unrelated non-selective ET_A/ET_B receptor antagonists on the contractions induced by ET-1 (ET_A/ET_B non-selective) and IRL 1620 (ET_B receptor selective) in the isolated upper bronchus of the guinea-pig (GPUB).

Male Dunkin-Hartley guinea-pigs (350-450 g) were killed by cervical dislocation and rapidly bled. The upper bronchi were excised, placed in Krebs-Henseleit buffer solution, trimmed of connective tissue and fat, cut into spirals and mounted in 10 ml organ baths and connected to auxotonic force-displacement transducers under tensions of 1.5 g. Tissues were equilibrated for 1 hour (with washing every 15 min) in gassed (95% O_2: 5% CO_2) Krebs-Henseleit solution (37°C, pH 7.4). Contractions are expressed as % of maximum histamine (5 μM) contraction. Ro 46-2005 (1, 10, 30, 100 μM) or PD 145065 (1, 10, 100 μM) were added to the baths 20 min before ET-1 or IRL 1620. Maximum contractions were defined as those to 3 x 10^{-7} M ET-1 or IRL 1620 and apparent EC_{50} values calculated accordingly.

Table 1. Effects of PD 145065 and Ro 46-2005 on the maximal responses (at 3 x 10^{-7} M) and apparent pD2 values for endothelin-1 or IRL 1620 in the isolated upper bronchus of the guinea-pig (*, $p < 0.05$).

	n	EC_{50}(nM)	-fold	E_{max}	% of ET-1
control (ET-1)	18	32±3	----	116±7	100
+PD 145065(10^{-5} M)	4	77±22	2	104±20	90
+PD 145065(10^{-4} M)	3	>1000	31	30±13*	26*
+Ro 46-2005 (3X10^{-5} M)	7	12±2	<1	131±8	113
+Ro 46-2005 (10^{-4} M)	3	29±11	<1	105±14	91
control (IRL 1620)	12	35±4	----	101±7	100 (87:ET-1)
+PD 145065 (10^{-6} M)	11	817±77	23	18±4*	18*
+PD 145065 (10^{-5} M)	6	>1000	29	0.6±0.3*	1*
+Ro 46-2005 (10^{-6} M)	5	83±25	2	89±14	88
+Ro 46-2005 (10^{-5} M)	4	325±95	9	49±7*	49*

PD 145065 (10^{-5} and 10^{-4} M) significantly attenuated the contractions induced by ET-1 whereas Ro 46-2005 (3 x 10^{-5} and 10^{-4} M) was without effect. PD 145065 (10^{-6} and 10^{-5} M) also strongly inhibited contractions induced by IRL 1620, whereas Ro 46-2005 caused much less antagonism (see Table 1). Thus, in the GPUB, PD 145065 is a more potent antagonist than Ro 46-2005 of contractions induced by ET-1 or IRL 1620 which are mediated by ET_B receptors.

We thank Parke-Davis Pharmaceutical Research for a grant, and the Medicinal Chemistry Department for providing Ro 46-2005 and PD-145065. BB is a recipient of Research Fellowship Awards from the National Heart and Stroke Foundation of Canada and the FCAR du Québec.

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117P PD 145065 BUT NOT BQ-123 BLOCKS BRONCHOCONSTRICTION INDUCED BY INTRAVENOUS ENDOTHELIN-1 OR SARAFOTOXIN 6c IN THE ANAESTHETISED GUINEA-PIG

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The endothelin/sarafotoxin (ET/SX) family of peptides are potent constrictors of airway preparations both *in vitro* and *in vivo*. In guinea-pigs intravenous injection of endothelin-1 (ET-1) produces bronchoconstriction due to the release of cyclo-oxygenase metabolites, particularly thromboxane A2 (Payne and Whittle, 1988). Two receptors for the ET/SX peptides have been identified. The ETA receptor, that is selective for ET-1 or sarafotoxin 6b and on which sarafotoxin 6c (SX6c) is largely without effect, and the non-isopeptide-selective ETB receptor. Using as agonists ET-1 and SX6c, and as antagonists BQ-123 (ETA receptor-selective) and PD 145065 (ETA/B receptor non-selective, Cody et al., 1992) we have characterised the receptors mediating bronchoconstrictions induced by the ET/SX peptides.

Male Dunkin-Hartley guinea-pigs (300-450 g) were anaesthetised with urethane (8 ml kg^{-1} of a 25% w/v solution, i.p.). The left carotid artery was cannulated for measurement of mean arterial pressure (MAP), the left jugular vein for the administration of drugs, and the trachea for ventilation (45 strokes min^{-1}, 6 ml kg^{-1}) and the measurement of pulmonary insufflation pressure (PIP). After 30 min the animals were treated with either indomethacin (indo, 5 mg/kg, i.v.) or vehicle (5 % $NaHCO_3$). After a further stabilisation period (30 min) BQ-123 or PD 145065 (both 10^{-6} mol kg^{-1}) or vehicle (0.1 % BSA in 0.9 % saline) was given as a bolus i.v. injection 3 min prior to ET-1 or SX6c. Four injections of ET-1 or SX6c were given at hourly intervals. The peak changes in PIP (mm Hg) after injection of ET-1 or SX6c are shown (mean±sem, n=4-5 for each group; *p<0.05; unpaired two-tailed t-test w.r.t. control) in the table:

Dose	ET-1				SX6c			
(nmol kg^{-1})	control	BQ-123	PD 145065	indo	control	BQ-123	PD 145065	indo
0.03	2±0.6	1±0.2	0±0*	0±0*	4±1.3	5±2.5	0±0.1*	0±0*
0.1	2±0.6	2±0.7	0±0.3*	0±0*	6±2.1	4±.7	0±0.1*	0±0*
0.3	4±0.9	3±0.6	1±0.4*	0±0*	10±2.1	6±0.9	1±0.3*	0±0*
1.0	5±1.5	9±2.4	2±0.6	0±0*	13±4.3	10±2.2	7±2.0	4±3.7*

Thus, in the guinea-pig i.v. ET-1 or SX6c cause bronchoconstrictions that are strongly antagonised by PD 145065 or indomethacin, but not by BQ-123. The ET/SX peptides, therefore, produce these effects via the formation of cyclo-oxygenase products secondary to activation of ETB receptors.

We thank Parke-Davis Pharmaceutical Research for funding this work, and the Chemistry Department for providing BQ-123 and PD 145065 (Ac-D-Bhg-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp), (D-Bhg = 5H-dibenzyl[a,d]cycloheptene-10,11-dihydro-glycine).

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118P CHARACTERISATION OF ENDOTHELIN RECEPTORS IN THE ISOLATED GALLBLADDER OF THE GUINEA-PIG

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Endothelin (ET-1) is a potent constrictor of smooth muscle including airways, the urinary bladder, the uterus, the stomach fundus, the ileum, the colon and the gallbladder. Two distinct endothelin receptors, ET_A (ET-1-selective) and ET_B (ET-1=ET-3), have been cloned. In airways from guinea-pigs, ETB receptors mediate contractions (Battistini et al., 1993). However, it is not known which receptor subtype(s) mediate these effects in gallbladders from guinea-pigs (GPGB; Moummi et al., 1992). Here we have used as agonists ET-1, ET-3, SX6c (a selective ETB agonist), and the ETB receptor selective analogues IRL 1620, BQ-3020, [$Ala^{1,3,11,15}$]-ET-1 and ET(16-21), and as antagonists BQ-123 (ETA receptor selective) and PD 145065 (ETA/ETB non-selective; Cody et al., 1993) to characterize the endothelin receptors in GPGB.

Male Dunkin-Hartley guinea-pigs (350-450 g) were killed and rapidly bled. The gallbladders were excised, placed in Krebs-Henseleit buffer solution, trimmed free of connective tissue and fat, and opened along their longitudinal axis. Two strips were obtained from each gallbladder by cutting parallel to the long axis of the organ and were mounted separately in 10 ml organ baths under tensions of 1.0 g and connected to Grass FT.03 force-displacement isometric transducers coupled to a Grass 79D recorder. Tissues were equilibrated for 1 hour (with washing every 15 min) in gassed (95% O2: 5% CO_2) Krebs-Henseleit solution (37°C, pH 7.4). Contractions are expressed as % of maximum histamine (5 µM) contraction. BQ-123 (10, 100 µM) or PD 145065 (10, 100 µM) were added to the baths 20 min before ET-1 or ET-3.

Table 1. Potency of ET/SX pepties and analogues in the GPGB

agonists	n	pD_2	-fold	E_{max}	% of ET-1
ET-1	7	7.65	------	230±25	100
ET-3	12	7.43	1.66	287±37	125
SX6c	12	7.80	0.71	95±9*	41*
IRL 1620	9	7.75	0.79	43±9*	19*
4*Ala*-ET-1	8	7.29	2.29	14±3*	6*
BQ-3020	5	6.95	5.01	25±8*	11*
ET (16-21)	10	>5.00	>50	3±2*	1*

ET-1 and ET-3 were equally active in producing contractions while ETB selective agonists induced less than 50 % of the maximal contractile effect to ET-1 at 3 x 10^{-7} M (Table 1). ET (16-21), up to 10 µM, did not induce contractions. BQ-123 (10, 100 µM) slightly inhibited contractions to ET-1 (2 and 6 fold shift to the right) but significantly blocked the response to ET-3 (>28 fold shift to the right). PD 145065 (10, 100 µM) significantly attenuated contractions induced by ET-1 (5 and 19 fold shift to the right) and blocked ET-3 even more effectively than BQ-123. This suggest that both ETA and ETB receptors mediate contractions to ET-1 and ET-3 in the GPGB.

Interestingly, ET-1 and ET-3 were approximately equipotent suggesting an ETB mediated response. Thus a classification based only on the potency of ET/SX agonists could be flawed. Also, the low efficacy of BQ-123 or PD 145065 in the GPGB compared to the potency of either antagonists in isolated vascular preparations may suggest the existence of different ET receptor subtypes.

We thank Parke-Davis Pharmaceutical Research, the Wellcome Trust and the Frances & Augustus Newman Trust for grants, and the Medicinal Chemistry Department of Parke-Davis for providing BQ-123 and PD 145065. BB is a recipient of Research Fellowship Awards from the National Heart and Stroke Foundation of Canada and the FCAR du Québec.

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119P ENDOTHELIN-1 (ET-1) ANTAGONISM OF VASCULAR RELAXATION IN HUMAN PULMONARY VESSELS BY MODIFICATIONS OF cGMP

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Vascular tone is maintained by an interaction of endothelium-derived contracting and relaxing agents (*LUSCHER et al., 1990; RUSSELL et ROBERT, 1991*). In pulmonary hypertension, the ET-1 plasma level and expression in endothelial cells are elevated (*GIAID et al.*, 1993) and endothelium-dependent relaxations are attenuated. The aim of this study was to examine the effects of ET-1 on the nitroprusside (SNP) induced relaxation and the cGMP increase in human isolated pulmonary arterial (HPA) and venous (HPV) preparations. Rings (2-3 mm internal diameter) were suspended in tissue baths (initial loads: 2.5g) containing Tyrode's solution at 37 °C gassed with 5% CO_2 in 95% O_2 for isometric tension recording. After equilibration (90 min), rings were contracted with either norepinephrine (NE) or ET-1 at the concentrations producing 50% (EC_{50}) and 100% (EC_{100}) of the maximal response. Relaxation curves were produced with SNP in the presence of indomethacin (1.7 µM) and N^{ω}-nitro-L-arginine (100 µM). Measurements of total cGMP level in SNP-stimulated HPA and HPV were conducted in the presence of indomethacin and N^{ω}-nitro-L-arginine. NE or ET-1 were incubated for 15 min before stimulation with SNP (100 µM, 4 min). Concentrations of cGMP were determined by enzymoimmunoassay. SNP increased the cGMP level by 10 fold in HPA and HPV. At 0.2 µM, ET-1 reduced significantly the cGMP increase in SNP stimulated vessels. At 0.02 µM, ET-1 induced a significant inhibition only in HPV. NE (0.1 or 10 µM) did not affected the cGMP increase in HPA or HPV. The sensitivity to SNP in HPV precontracted with 0.2 µM of ET-1 (EC_{50} = 0.046 ± 0.014 µM) was significantly ($P < 0.05$) diminished *versus* 0.02 µM (EC_{50} = 0.66 ± 0.35 µM). This alteration correlated with the inhibition of the cGMP increase in SNP stimulated HPV. The response to SNP did not differ when the arteries were precontracted with ET-1 (0.02 or 0.2 µM). In addition, SNP completely reversed the contraction of rings treated by NE (0.1 or 10 µM).

cGMP concentration in SNP stimulated human pulmonary vessels

	SNP (100 µM)	N	HPA (pmol/mg)	HPV (pmol/mg)
control	-	9	2.50 ± 0.38	3.43 ± 1.03
control	+	9	21.24 ± 10.61	30.43 ± 12.45
ET-1 0.02 µM	+	6	10.68 ± 3.36	9.63 ± 3.61 *
ET-1 0.2 µM	+	6	8.79 ± 2.35 *	5.42 ± 1.60 *

cGMP level was expressed as pmol per milligram of protein. N = number of lung samples and (*), values significantly different ($P < 0.05$; Student's t-test) from SNP stimulated vessels.

The data show that ET-1 can induce a functional antagonism in human pulmonary vessels suggesting that ET-1 may play a role in the control of muscle tone in human pulmonary vascular bed.

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120P THREE ENDOTHELIN RECEPTOR SUBTYPES SUGGESTED BY THE DIFFERENTIAL POTENCY OF BOSENTAN, A NOVEL ENDOTHELIN RECEPTOR ANTAGONIST, IN ISOLATED TISSUES

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Two endothelin (ET) receptors have been cloned and characterised, the ET_A receptor which shows selectivity for ET-1 and ET-2 relative to ET-3 and the ET_B receptor which is non-isopeptide selective. ET_A receptors mediate constriction of vascular smooth muscle while ET_B receptors can mediate both vasodilation via liberation of relaxant factors from the endothelium, and vasoconstriction via receptors located on smooth muscle cells. Ro 47-0203 (bosentan, 4-tert-butyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2'-bipyrimidin-4-yl]-benzenesulfonamide) is a novel competitive antagonist of ET_A and ET_B receptors derived from the previously described antagonist Ro 46-2005 (Clozel *et al.*, 1993). The present study investigated the ability of Ro 47-0203 to differentiate between receptors mediating contraction and relaxation in isolated tissue preparations.

The antagonist potency of Ro 47-0203 was determined on functional responses mediated by the ET_A receptor (contraction of the rat aorta by ET-1), by the endothelial ET_B receptor (relaxation of preconstricted rat small mesenteric artery and rabbit superior mesenteric artery by the selective ET_B receptor agonist, sarafotoxin (SRTX) S6c) and by the smooth muscle ET_B receptor (constriction of rabbit saphenous vein and rat trachea by SRTX S6c). Vascular or tracheal tissue was removed from anaesthetised male New Zealand White rabbits and Wistar Kyoto rats, cut into rings and mounted in organ baths containing warmed (37°C) and gassed (95% O_2/5% CO_2) Krebs-Henseleit solution. Responses to ET-1 and SRTX S6c were determined after 20 min incubation with 0.3 - 10µM Ro 47-0203 or its solvent, DMSO. The pA_2 value, as an index of antagonist potency, was determined by regression analysis of the Schild plot.

Ro 47-0203 had no partial agonist activity *per se* but was a competitive antagonist at both ET_A and ET_B receptors (slope of the Schild plot was not significantly different from 1). The order of antagonist potency was ET_A (pA_2 of 7.4 ± 0.1, n = 15) > endothelial ET_B (pA_2 of 6.8 ± 0.1, n = 13 on rat small mesenteric arteries and 6.7 ± 0.1, n = 14 on rabbit superior mesenteric artery) > smooth muscle ET_B (pA_2 of 6.1 ± 0.1 , n = 15 on rabbit saphenous vein and 6.0 ± 0.1, n = 28 on rat trachea).

Thus, Ro 47-0203 is able to distinguish not only between ET_A and ET_B receptor subtypes but also between the smooth muscle and endothelial ET_B receptors. The difference in antagonist potency, whichis similar to that found using a non-subtype selective peptide endothelin receptor antagonist (Warner *et al.*, 1993), is strongly suggestive of the existence of subtypes of the ET_B receptor or at least a difference in the conformational state of the two receptors.

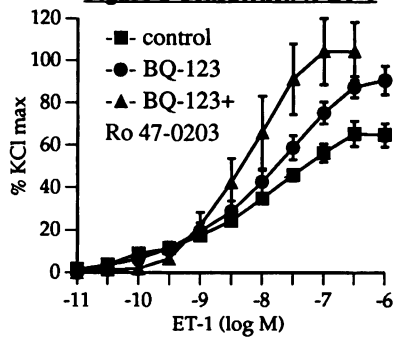
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121P ET-1 ACTIVATES MULTIPLE RECEPTOR SUBTYPES, INCLUDING AN INHIBITORY ET_A RECEPTOR, IN THE RAT FUNDIC STRIP

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Two subtypes of receptor for endothelin (ET) have been identified, the ET_A receptor which shows selectivity for ET-1 and ET-2 relative to ET-3 and the ET_B receptor which is non-isopeptide selective. In the rat fundus ET-1 can produce constriction and relaxation (Olsen and Weis, 1992). The present study investigated the role of ET_A and ET_B receptors in these responses using ET-1, sarafotoxin (SRTX) S6c, a selective agonist of ET_B receptors; BQ-123, a selective ET_A receptor anatagonist and Ro 47-0203 (bosentan, 4-tert-butyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2'-bipyrimidin-4-yl]-benzenesulfonamide) a novel antagonist of ET_A and ET_B receptors derived from the previously described antagonist Ro 46-2005 (Clozel *et al.*, 1993). Rat fundic strips were suspended under 1 g tension in organ baths containing 10 ml of warmed (37°C) and gassed (95% O_2/5% CO_2) Krebs-Henseleit solution. Cumulative dose-response curves to ET-1 and SRTX S6c were obtained in the absence and presence of BQ-123 (10μM) and/or Ro 47-0203 (10μM). Responses are expressed as a percentage of the response obtained to 50mM KCl prior to agonist dose-response curves.

Figure 1 Constriction to ET-1



Both ET-1 (Fig 1) and SRTX S6c produced dose-dependent contraction of fundic strips, although the E_{max} for SRTX S6c (86 ± 4 % KCl, n=8) was significantly ($p<0.02$) higher than that of ET-1 (65 ± 6 % KCl). In the presence of BQ-123 the E_{max} of ET-1 was significantly increased (Fig 1). Ro 47-0203 inhibited responses to low concentrations of both ET-1 (Fig 1) and SRTX S6c, but significantly increased the maximal response obtained with both, in the presence and in the absence of BQ-123, Neither BQ-123 nor Ro 47-0203 had any partial agonist activity in this preparation.

Induction of contraction by SRTX S6c demonstrates the presence of "contracting type" ET_B receptors in the rat fundic strip. Although ET_A receptors are normally associated with contraction, potentiation of ET-1 responses by BQ-123 suggests that their primary role in the fundic strip is to oppose contraction. Inhibitory ET_B receptors also appear to have a role since the non-selective antagonist Ro 47-0203 was able to enhance contraction elicited by SRTX S6c and by ET-1 in the presence of BQ-123. The atypical inhibitory effect of ET_A receptor stimulation may indicate the existence of a novel ET_A receptor subtype in the rat fundic strip.

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122P CHARACTERISATION OF THE ENDOTHELIN ET_A ANTAGONISTS BQ123 AND FR139317 IN HUMAN, RAT AND PORCINE HEART, AND THE ET_B-SELECTIVE ANTAGONIST IRL1038 IN HUMAN TISSUE

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We have compared the ability of ET_A-selective antagonists FR139317 (modified tripeptide; Urade et al., 1992) and BQ123 (cyclic pentapeptide; Davenport et al., 1993), as well as the putative ET_B-selective antagonist IRL1038 ([Cys^{11}-Cys^{15}]-ET-1(11-21); Aramori, I., et al. 1993) to compete with radiolabeled [^{125}I]ET-1binding to human, rat and porcine left ventricle.

For competition binding, slide mounted tissue sections were incubated for 120 minutes in HEPES buffer (50 mM HEPES, 5 mM $MgCl_2$, and 0.3% bovine serum albumin, pH 7.4) at room temperature, containing 0.1nM [^{125}I]ET-1 (Amersham International) with or without concomitant addition of the competing unlabeled antagonists (1pM-100μM). Non-specific binding was defined by ET-1 (1μM). Binding parameters were calculated using the iterative curve fitting programme LIGAND.

Antagonist	Tissue	K_D ET_A [nM]	K_D ET_B [μM]	B_{max} ET_A [fmol/mg]	B_{max} ET_B [fmol/mg]
BQ123	human *	0.73 ± 0.22	24.3 ± 2.0	56.5 ± 5.1 (57%)	42.0 ± 1.6 (43%)
	porcine	0.52 ± 0.05	70.4 ± 4.0	414 ± 38 (69%)	186 ± 11 (31%)
	rat	1.18 ± 0.16	1370 ± 1150	46.2 ± 1.7 (67%)	20.7 ± 1.8 (33%)
FR139317	human	1.20 ± 0.28	287 ± 93	35.7 ± 2.3 (62%)	21.9 ± 1.9 (38%)
	porcine	2.17 ± 0.51	47.1 ± 5.7	657 ± 3 (64%)	366 ± 3 (36%)
	rat	2.28 ± 0.30	292 ± 114	45.2 ± 2.3 (62%)	27.2 ± 2.2 (38%)
IRL1038	human	37600 ± 33500	5.76 ± 8.19	30.8 ± 2.2 (62%)	19.2 ± 2.8 (38%)

These data (n≥3, mean ± s.e.mean) show that the modified linear tripeptide FR139317 can compete for the binding of [^{125}I]ET-1 in human heart. Both compounds are highly selective for ET_A subtype in all three heart tissues (range 10^4-10^6 fold compared to ET_B). They may provide useful structural information for the synthesis of novel non-peptide antagonists. IRL1038 shows only a 10-fold selectivity for ET_B receptors with a K_D of 5.8μM making it less effective as selective antagonist at least in ET_A-rich tissues such as human left ventricle (appr. 60% ET_A-receptor subtypes).

We are grateful to the Theatre Staff at Papworth Hospital for permission to collect human tissue samples and we thank the Shering Research Foundation and SERC for their support.

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123P ENDOTHELIN STIMULATION OF MITOGENESIS IN VASCULAR SMOOTH MUSCLE FROM HYPERTENSIVE RATS: RELATIONSHIP TO PHOSPHOLIPASE D

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Vascular smooth muscle from spontaneously hypertensive rats (SHR) has been reported to show an enhanced mitogenic reponse to a variety of stimuli compared with their normotensive controls (WKY). We have recently provided evidence that phospholipase D may be involved in the mitogenic response of vascular smooth muscle cells to endothelin 1 (ET-1) (Wilkes et al, 1993). Here we report experiments comparing the stimulation of mitogenesis, phospholipase C (PLC) and phospholipase D (PLD) in SHR and WKY derived vascular smooth muscle cells .

Primary smooth muscle cell cultures were established from aortae of 12 week old SHR and WKY rats and used within 15 passages. For determination of [^{3}H]thymidine incorporation into nucleic acid cells were maintained serum free for 24h followed by stimulation with ET-1 for 24h. During the last 4h [^{3}H]thymidine (0.5 μCi ml^{-1}) was added, following which cells were washed and nucleic acids extracted. For measurement of mass of inositol 1,4,5-trisphosphate (IP_3) a protein binding assay was used. For measurement of PLD [^{32}P]phosphatidylbutanol was estimated in cells loaded with ^{32}Pi and stimulated in the presence of butanol (Purkiss and Boarder, 1992). Data are presented as mean ± s.e.mean (n = 3).

ET-1 at 100 nM failed to stimulate [^{3}H]thymidine incorporation into WKY derived cells (control 0.43 ± 0.03; ET-1 0.38 ± 0.10; dpm x 10^{-3} per well). However [^{3}H]thymidine incorporation into SHR derived cells was stimulated (control 1.92 ± 0.17; ET-1 10.26 ± 0.47; dpm x 10^{-3} per well). ET-1 stimulated IP_3, the primary product of phosphoinositide specific PLC, was studied between 5 s and 5 min. Both SHR and WKY cells responded with a clear stimulation; however the effects of 100 nM ET-1 was considerably larger with the SHR cells. For example with 30 s stimulation: WKY cells, control 9.31 ± 0.16, ET-1 28.41 ± 8.45; SHR cells, control 16.04 ± 2.99, ET-1 199.46 ± 22.64; fmols per μg protein). When [^{32}P]phosphatidylbutanol was measured the comparison of SHR and WKY cells was the opposite of that expected; the SHR response to 100 nM ET-1 was smaller than the WKY response. For example with 2 min stimulations: WKY cells, control 9.68 ± 3.20, ET-1 92.79 ± 4.33 ; SHR cells, control 6.53 ± 1.87, ET-1 24.27 ± 2.13; dpm x 10^{-3} per mg protein. Pooled across 3 experiments, with basal values deducted, SHR cells gave 22.7 ± 3.24, while WKY cells gave 79.17 ± 3.01, dpm x 10^{-3} per mg protein.

In summary these observations show that while SHR derived cells have an enhanced mitogenic and PLC response to 100 nM ET-1, they exhibit an attenuated PLD response.

This work was supported by The Wellcome Trust, The MRC, and The Nuffield Trust.

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124P MECHANISM OF RESPONSES TO BRADYKININ IN HUMAN ISOLATED PERIPHERAL AIRWAYS

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The pro-inflammatory peptide bradykinin causes bronchoconstriction in asthmatics, but not in normals (Fuller *et al.*, 1987). The mechanism of responses induced by bradykinin, however, remains unclear. Studies *in vivo* in man suggest that bradykinin causes cholinergic activation, release of prostanoids, and local axon reflexes with release of tachykinins (Fuller *et al.*, 1987; Ichinose *et al.*, 1992). We investigated the effects of cyclooxygenase inhibition (indomethacin; 5 x 10^{-7} M), neural conductance blockade (tetrodotoxin; 3 μg/mL), and the selective bradykinin B_2 receptor antagonist Hoe 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin; Hock *et al.*, 1991) on responses to bradykinin of human isolated peripheral airways (internal diameter 0.8 to 2 mm). We also compared the responses to bradykinin of airways with or without epithelium. Finally, we measured the bradykinin-induced release of the prostanoids PGE_2, PGI_2, and TxA_2 from airways with or without epithelium in the absence or presence of indomethacin by radioimmunoassay. Airways were obtained from patients operated because of bronchial carcinoma (age: 48-77 years) and responses were recorded isotonically in organ baths containing carbogenated Krebs-Henseleit buffer and phosphoramidon, an inhibitor of neutral endopeptidase. Concentration response curves to bradykinin (10^{-10} to 10^{-5} M) were obtained and sensitivity was expressed as -log EC_{50}. Data were compared with paired Student's *t* test (α=0.05) and expressed as means ± SEM. All airways were contracted by bradykinin. Removal of the epithelium significantly increased the sensitivity to bradykinin (-log EC_{50}: 7.14 ± 0.21 and 8.04 ± 0.13 for airways with and without epithelium, respectively, n=8). Treatment with indomethacin abolished responses to bradykinin and release of the prostanoids PGE_2, PGI_2, and TxA_2 in both airways with and without epithelium (n=8). The release of prostanoids was greater in airways without epithelium indicating that human airway smooth muscle can produce these substances. The sensitivity to bradykinin in the presence of tetrodotoxin was not significantly different from the control (-log EC_{50}: 7.26 ± 0.20 and 7.32 ± 0.21 in the presence and absence of tetrodotoxin, respectively, n=8). Hoe 140 produced a significant rightward shift of the concentration response curves to bradykinin (-log EC_{50} values: 7.28 ± 0.14, 6.75 ± 0.12, 6.31 ± 0.17 and 5.54 ± 0.13 for controls, 10^{-8}, 10^{-7} and 10^{-6} M Hoe 140, respectively, n=6). The Schild plot showed an apparent pA_2 of 8.3. However, the slope was 0.76 ± 0.02 probably indicating that Hoe 140 is a noncompetitive antagonist. We conclude that bradykinin is a potent constrictor of human peripheral airways, particularly in the absence of epithelium. Responses to bradykinin in human peripheral airways are mainly mediated via the B_2 receptor and involve the release of prostanoids. Involvement of local axon reflexes could not be demonstrated. Since epithelial damage is a characteristic of asthma and bradykinin concentrations are elevated in bronchoalveolar lavage fluid of asthmatics after allergen challenge, endogenous bradykinin may indeed contribute to bronchoconstriction in asthmatics.

Study supported by *The Netherlands Asthma Foundation*, grant 90.43.

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125P BRADYKININ-STIMULATED PtdIns(4,5)P_2 HYDROLYSIS BY PHOSPHOLIPASE C IS NOT A PRE-REQUISITE FOR PtdCho HYDROLYSIS BY PHOSPHOLIPASE D OR C IN AIRWAY SMOOTH MUSCLE

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A novel B_3 bradykinin (BK) receptor has been proposed to exist in cultured guinea pig tracheal smooth muscle (TSM) cells (Farmer *et al.*, 1991). We have previously shown that BK stimulates the PLD-catalysed hydrolysis of phosphatidylcholine (PtdCho) in a protein kinase C (PKC)-dependent manner in these cells (Pyne & Pyne, 1993a). Here we demonstrate that this response is independent of B_1 and B_2 receptors and that PLC-catalysed PtdIns(4,5)P_2 hydrolysis is not a prerequisite of PLD activation (Pyne & Pyne, 1993b).

TSM cells were isolated and maintained in culture for 14-21 days prior to experiment. BK-stimulated PLC-catalysed PtdIns(4,5)P_2 hydrolysis was assayed by Ins(1,4,5)P_3 mass measurement (Palmer & Wakelam, 1990), PLD-catalysed PtdCho hydrolysis by the accumulation of [^{3}H]phosphatidylbutanol ([^{3}H]PtdBut) in [^{3}H]palmitate-labelled cells, stimulated in the presence of butan-1-ol (0.3%, v/v) (Pyne & Pyne, 1993a) whereas accumulation of [^{3}H]1,2-diacylglycerol (DG), [^{3}H]1,3-DG and [^{3}H]phosphatidic acid (PtdOH) in [^{3}H]palmitate-labelled cells in the presence of butan-1-ol was used as a measure of PLC-catalysed PtdCho hydrolysis.

BK-stimulation elicited the sustained formation of [^{3}H]PtdBut, [^{3}H]1,2-DG, [^{3}H]1,3-DG and [^{3}H]PtdOH in [^{3}H]palmitate-labelled cells in a dose-dependent manner with log EC_{50} values of -8.3 ± 0.4 M, -8.29 ± 0.26 M, -8.3 ± 0.65 M and -8.8 ± 0.29 M (n=3, t=10 min), respectively, which were not significantly different from each other. BK also induced the rapid and transient formation of Ins(1,4,5)P_3 with a significantly lower potency (log EC_{50} of -7.55 ± 0.1 M (n=3, t=10 sec, $p < 0.05$). The BK B_1 agonist, desArg^9-BK failed to elicit either PLC response nor a PLD response. BK(10 nM, EC40)-stimulated Ins(1,4,5)P_3 formation was inhibited by a B_2 receptor antagonist (NPC 349 (D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]BK) with log IC_{50} of -6.3 ± 0.4 M (n=3) whereas BK(10 nM, EC_{100})-stimulated [^{3}H]PtdBut accumulation was poorly inhibited with $IC_{50} > 40$ μM, at which concentration the Ins(1,4,5)P_3 response was abolished. Similarly, BK-stimulated formation of [^{3}H]1,2-DG, [^{3}H]1,3-DG and [^{3}H]PtdOH were not inhibited by the B_2 receptor antagonist or elicited by the B_1 agonist. These results suggest that (i) BK elicits the B_2 receptor mediated, PLC-catalysed hydrolysis of PtdIns(4,5)P_2; (ii) BK-stimulated hydrolysis of PtdCho by phospholipases C and D is independent of B_2 receptors. Furthermore, since BK-stimulated activation of PLD is 'down-stream' of PKC (Pyne & Pyne, 1993a) and independent of PtdIns(4,5)P_2 hydrolysis, PLC-catalysed PtdCho hydrolysis may supply the PKC activator required to initiate PLD activity.

This study was supported by The Wellcome Trust (032585/Z/90/Z/1.5; 037892/Z/93/Z/1.4P)

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126P EFFECTS OF THE BRADYKININ ANTAGONIST, ICATIBANT (Hoe 140), ON THE ACTIONS OF THE VENOM FROM THE WASP *VESPULA VULGARIS*

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Several bradykinin-related peptides have been identified in wasp venoms (Schachter, 1968). The bradykinin B_2 receptor antagonist, icatibant (DArg-[Hyp^3-Thi^5-DTic^7-Oic^8]-bradykinin; Lembeck *et al.*, 1991; formerly named Hoe 140), was tested for its effects on *in vitro* and *in vivo* actions of the venom from the wasp, *Vespula vulgaris*.

The venom (0.2–4 μg ml^{-1}) caused concentration-dependent contractions of the isolated guinea-pig ileum tested in the presence of mepyramine (0.35 μM), ketanserin (1.0 μM) and atropine (0.35 μM). Icatibant (3–100 nM) caused a not competitive inhibition of the contractions induced by the venom or by bradykinin. A deviation from competitive antagonism by icatibant in this tissue has already been described earlier (Griesbacher & Lembeck, 1992). The inhibition curves for icatibant against half-maximal concentrations of the venom (4 μg ml^{-1}) and bradykinin (10 nM) were identical and yielded pIC_{50} values of 8.3 ± 0.1 and 8.5 ± 0.1, respectively.

Both bradykinin (0.3–30 nmol) and the venom (0.3–30 μg) caused a dose-dependent paw oedema after subplantar injection in barbiturate-anaesthetized rats. The net increases in paw volume 30 min after injection of the venom (10 μg) or bradykinin (30 nmol) amounted to 44 ± 6 % and 50 ± 6 %, respectively. Pretreatment with icatibant (300 nmol kg^{-1}, s.c.) reduced these increases in paw volume only to 33 ± 8 % and 36 ± 4 %, respectively ($P<0.05$). Additional pretreatment with mepyramine (20 μmol kg^{-1}, s.c.) did not cause a further significant attenuation of the oedema.

The subplantar injection of the venom (50 μg) or bradykinin (50 nmol) in unanaesthetized rats caused an increase in sensitivity scores (Cohen *et al.*, 1984) during the initial 15–20 min following the injection. Thereafter, no increase in sensitivity was observed. Although pretreatment with icatibant (300 nmol kg^{-1}, s.c.) had only a limited effect on the oedema, it completely prevented ($P<0.05$) the nociceptive responses to the venom or to bradykinin.

Conclusion: The kinins contained in the venom of *Vespula vulgaris* act on bradykinin B_2 receptors and are antagonized by icatibant in the same manner as synthetic bradykinin. The increase in plasma permeability induced by the venom rests partly on the actions of the wasp kinins; the resulting oedema is further augmented by the release of other mediators. The activation of nociceptive nerve fibres following challenge with the venom solely relies on the action of the kinins contained in the venom and can be prevented completely by icatibant.

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127P THE LACK OF INHIBITION OF CAPSAICIN-INDUCED OEDEMA BY DEXAMETHASONE

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The anti-inflammatory steroid, dexamethasone, inhibits plasma protein extravasation induced by intradermal (i.d.) mediators of increased microvascular permeability in rat and rabbit skin (Ahluwalia *et al.*, 1992; Yarwood, *et al.*, 1993). Capsaicin, which selectively activates sensory nerves, stimulates an increase in blood flow and, at higher doses, oedema formation in rabbit skin (Buckley *et al.*, 1990). In this study we have investigated the effect of systemic dexamethasone pretreatment on neurogenic oedema induced by i.d. capsaicin in the skin of rabbits.

Male rabbits (2-3kg) were clipped of dorsal fur and anaesthetized with Sagatal (30mgkg^{-1} i.v.). Oedema formation was measured by the local accumulation of ^{125}I-labelled human serum albumin (Buckley *et al.*, 1990). Test agents (0.1ml i.d.) were injected according a balanced site pattern with six replicates per test agent. After an accumulation period of 30 minutes the animal was killed by overdose. Skin sites were counted in a gamma counter together with 1ml plasma samples. Plasma protein extravasation was expressed in µl by dividing counts in each skin site by counts in 1µl of plasma.

Table 1. Effect of dexamethasone (Dex 3mgkg^{-1} i.v. -4h) on oedema (µl plasma) induced by capsaicin and bradykinin (BK) in the presence and absence of ruthenium red (R.R.). Mean ± s.e.mean, n=6 for each data set. Statistics by two way ANOVA and Bonferroni's modified t-test.

	Pretreatment (i.v. -4h)				Pretreatment (i.v. -4h)		P
Test Agent	Saline	Dex	P	Test Agent	Saline	Dex	
Saline	6.2±0.8	4.2±0.8	NS	R.R. 10nmol	4.8±0.5	3.6±0.5	NS
Capsaicin 3µmol	38.7±4.4	42.8±9.0	NS	Capsaicin 3µmol+ R.R. 10nmol	12.7±0.6	8.2±1.1	NS
BK 1nmol	36.6±3.4	12.3±1.0	<0.01	BK 1nmol+ R.R. 10nmol	30.4±3.4	13.5±1.4	<0.01

The inhibition of capsaicin-induced oedema but not BK-induced oedema by R.R. suggests that capsaicin induces oedema by activation of the putative capsaicin receptor operated non-selective cation channel and/or neuropeptide release. The results show that capsaicin-induced oedema in rabbit skin is resistant to the anti-inflammatory activity of dexamethasone.

P.Newbold is a SERC student. We thank Fisons for support.

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128P [α-METHYL-TYROSINE^2]-PROCTOLIN BLOCKS PROCTOLIN RECEPTORS IN THE LOCUST FOREGUT

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Proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH), first identified by Brown (1967), has been shown to cause dose-dependent contraction of the isolated foregut of the locust *Schistocerca gregaria* (Banner *et al.*, 1987a). Structure activity studies have shown that slight modification to the peptide sequence resulted in an almost complete loss of activity (Starratt & Brown, 1979) although substituting the hydroxyl of the tyrosine with a methoxy group produced an analogue with three times the potency of proctolin. Subsequent research into the physiology of this peptide has been hampered by the lack of specific and reliable antagonists. This report describes the effects of four new analogues one of which is a potent antagonist of proctolin.
Isolated foreguts (oesphagus to proventriculus) were incubated in Clarke Insect Ringer at 18±2°c for twenty min. prior to testing the contractile effects of proctolin and a range of its analogues using an isotonic recording system. All analogues were dissolved in Clarke Insect Ringer and using a six minute cycle with two washes, attempts were made to construct dose response curves for proctolin, [N-methyl-threonine^5]-proctolin, [N-methyl-leucine^3]-proctolin, [N-methyl-tyrosine^2]-proctolin and [α-methyl-tyrosine^2]-proctolin.

Proctolin caused dose-dependent tissue contraction with an ED_{50} value of 1.15 ± 0.1 x 10^{-7}M at doses ranging from 10^{-8}M to 2 x 10^{-6}M (n = 60). The ED_{50} values for [N-methyl-threonine^5]- proctolin and [N-methyl-leucine^3]-proctolin were 3 x 10^{-7}M (n = 7) and 8 x 10^{-5}M (n = 7) respectively but, their intrinsic activities were only 78% and 52% respectively of that of proctolin. [N-methyl-tyrosine^2]-proctolin and [α-methyl-tyrosine^2]-proctolin were without agonist action. at concentrations ranging from 10^{-9}M to 10^{-4}M, and subsequently, both peptides were tested for antagonist activity. Tissues were incubated with either peptide, at concentrations ranging from 10^{-8}M to 10^{-5}M, for 20 min. prior to retesting the effects of proctolin in their presence. Once again, the [N-methyl tyrosine^2] derivative was without effect but, [α-methyl-tyrosine^2]-proctolin caused dose-dependent non competitive antagonism significantly reducing the maximum response to applied proctolin by 45% (10^{-8}M, n = 8, P<0.005), 73% (10^{-7}M, n = 8, P<0.0005) and 88% (10^{-6}M, n = 8, P<0.0005). The demonstration that [α-methyl-tyrosine^2]-proctolin is a potent proctolin antagonist means that it is now possible to characterize the receptors activated by the parent compound as well as elucidating their physiological and biochemical roles in insect tissues.

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129P NICORANDIL DILATES LARGE EPICARDIAL CORONARY ARTERIES IN CONSCIOUS DOGS THROUGH A NITRATE-LIKE, ENDOTHELIUM-INDEPENDENT MECHANISM

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The vasorelaxant activity of nicorandil, a new antianginal drug, appears to depend upon two major mechanisms, *i.e.*, a nitrate-like action and a potassium channel opener (KCO)-like action (Meisheri *et al.*, 1991). The aim of the present investigation was to uncover the pharmacological mechanism by which nicorandil (N) produces dilation of large coronary arteries in conscious dogs and to determine the relationship between this vasodilator effect and the coronary endothelium. For this purpose, we compared the coronary vasodilator properties of N to those of nitroglycerin (GTN), a pure nitrate derivative, and pinacidil (P), a pure KCO, in the presence and absence of an intact endothelium in the conscious dog.

Six dogs were instrumented under sterile conditions for measurement of large circumflex coronary artery diameter (CD, piezoelectric crystals) and coronary blood flow (CBF, Doppler). Coronary vascular resistance (CVR), an index of reactivity of small coronary arteries, was calculated as mean arterial pressure (MAP, intra-aortic catheter) / CBF ratio. Three weeks after the initial surgery, each dog, in the conscious state, received on separate days N (30 to 300 μg/kg), GTN (0.3 to 3 μg/kg) and P (10 to 100 μg/kg) (i.v. bolus) in a random order. In addition, coronary dilation in response to acetylcholine (A) (0.1 to 1.0 μg/kg) and reactive hyperemia after the release of a 20-sec coronary occlusion (RH) (n=5) were assessed. Three days after mechanical removal of the coronary endothelium by a ballon angioplasty technique (Drieu La Rochelle *et al.*, 1992), responses to N (100 μg/kg), GTN (1.0 μg/kg), P (30 μg/kg), A (0.3 μg/kg) and RH were evaluated. Values are expressed as mean$\pm$s.e.mean.

N, GTN and P induced similar decreases in CVR before and after endothelium removal. At the level of large coronary arteries, N, GTN and P induced a dose-dependent increase in CD before endothelium removal. Three days after endothelium removal, CD increase in response to A and RH were almost abolished (-81.4$\pm$3.6% and -90.8$\pm$3.7%, $p<0.001$, respectively), indicating the absence of a functional endothelium. P-induced dilation was strongly decreased (-75.9$\pm$4.9%, $p<0.01$). In contrast, vasodilation of the large coronary artery in response to N and GTN were only slightly reduced after endothelium removal and at the same extent (-19.4$\pm$4.0% and -28.2$\pm$9.3%, $p<0.01$ and $p<0.05$, respectively).

We conclude that nicorandil dilates large coronary arteries through an endothelium-independent mechanism, *i.e.*, a nitrate-like mechanism and that KCO properties are not involved in this effect.

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130P MECHANISM OF ANTIFIBRILLATORY ACTION OF THE INWARDLY RECTIFYING POTASSIUM CURRENT BLOCKER, RP58866, ASSESSED USING THE RAT DUAL CORONARY PERFUSION MODEL

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It has previously been shown that RP58866, a selective blocker of the inwardly rectifying potassium (K^+) current (I_{K1}; Escande *et al.*, 1992), is an extremely effective antiarrhythmic agent in rat, rabbit and primate (marmoset) isolated hearts in the settings of acute ischaemia and reperfusion (Rees & Curtis, 1993). In the present study we have examined the role of action potential widening in involved (ischaemic) versus uninvolved (non-ischaemic) tissue in mediating these antiarrhythmic effects.

We have made use of the new dual coronary perfusion cannula that allows left and right sides of the heart to be perfused independently (Avkiran & Curtis, 1991). Isolated rat hearts (n=112) were randomised to one of four groups: perfusion of the left and right coronary beds with drug-free solution (n=28), perfusion of the left coronary bed with 3 μM RP58866 (n=28), perfusion of the right coronary bed with 3 μM RP58866 (n=28) or perfusion of left and right coronary beds with 3 μM RP58866 (n=28). After 5 min, left regional ischaemia was induced in each heart and was maintained for 30 min by closing off completely the supply of perfusion solution to the left coronary bed. Regional coronary flow was measured by in-line flowmeters. Epicardial monophasic action potentials (MAP) were recorded from the left (n=15/group) and right (n=13/group) perfusion regions using a suction electrode.

The presence of RP58866 in the uninvolved zone was necessary for suppression of ischemia-induced ventricular fibrillation (VF); incidences (%) of VF were 80, 60, 33 ($p<0.05$) and 27 % ($p<0.05$) in groups with no drug, with RP58866 in the left bed, with RP58866 in the right bed and with RP58866 in the left plus right beds, respectively. Likewise, widening of MAP duration in the uninvolved zone was necessary for suppression of VF; uninvolved zone MAP duration at 100 % repolarization after 5 min of ischaemia (just prior to the onset of ventricular arrhythmias) was 130.6$\pm$8.0. 129.1$\pm$7.0, 155.8$\pm$6.5 ($p<0.05$) and 155.3$\pm$8.7 ($p<0.05$) in the four groups, respectively.

The specific I_{K1} blocker RP58866 prevents ischaemia-induced VF in rat by widening monophasic APD in the uninvolved zone. This action may give RP58866 a potential advantage over other K^+ channel blockers which prevent arrhythmias via actions in the ischaemic zone, since such agents commonly show diminished activity in such tissue (Cobbe, 1988).

S.A.R. is a Beit Memorial Fellow. Thanks to the BPS for funding this work (via an A.J. Clark Award to S.A.R).

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131P A NOVEL BIS-QUATERNARY CYCLOPHANE, UCL 1530, MAY DISCRIMINATE BETWEEN SUBTYPES OF APAMIN SENSITIVE K+ CHANNELS

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Several subtypes of Ca^{2+} activated K^+ channels have been identified on the basis of single channel conductance and sensitivity to blocking agents. Of these, the SK_{Ca} channel is characterized by a small single channel conductance (6-20pS) and high sensitivity to apamin ($K_d \approx$ 1nM). Although there have been some indications that apamin-sensitive channels differ between tissues, in general they have been regarded as a single class. In this study, we have compared the ability of dequalinium and a novel compound 8,19-diaza-3(1,4),5(1,4)-dibenza-1(1,4),7(1,4)-diquinolina-cyclononadecanephanedium(UCL 1530) to block the apamin-sensitive K^+ channel in rat sympathetic neurones, guinea-pig hepatocytes, and rabbit jejunum.

Blockade of the SK_{Ca} channels in rat sympathetic neurones was determined from the ability of compounds to inhibit the slow afterhyperpolarization that follows the action potential (see Dunn et al., 1991). Compounds were also tested for effects on Ca^{2+} currents recorded using the whole cell patch clamp technique, and on the direct activation of the SK_{Ca} channels by dialysing the cytosol with a Ca^{2+} from a patch electrode. Guinea-pig hepatocytes were isolated and angiotensin II-evoked K^+ loss through SK_{Ca} channels measured by K^+ sensitive electrodes as described by Cook and Haylett (1985). Inhibition of SK_{Ca} channels in the rabbit jejunum was measured by the ability of the compounds to block the relaxant effect of ATP (see Gater et al., 1985). UCL 1530 inhibited the K^+ efflux from guinea-pig hepatocytes with an IC_{50} of 2.8 ± 0.5 μM (fitted value ± S.D. 4 expts.) compared with 1.7 ± 0.3 μM for dequalinium. On the rabbit jejunum, 3μM dequalinium reduced the relaxant effect of 1-3 μM ATP by 70 ± 12 % (mean ± s.e. mean, n=3), while the same concentration of UCL 1530 produced 40 ± 6 % inhibition. The slow afterhyperpolarization recorded from sympathetic neurones was inhibited by dequalinium and UCL 1530 with IC_{50} values of 0.6 ± 0.2 and 0.08 ± 0.02 μM respectively (data from 10 cells). At a concentration of 1μM, UCL 1530 failed to reduce the Ca^{2+} current in these cells (n=3), whereas at 0.2μM it produced 76% inhibition of the hyperpolarization evoked by dialysis of neurones with high Ca^{2+} solution (n=3).

Hence, while UCL 1530 is less potent than dequalinium as a blocker of the apamin-sensitive SK_{Ca} channel in rabbit jejunum and guinea-pig hepatocytes, it is much more potent than dequalinium at blocking the channel present in sympathetic nerve cells. These results provide further evidence for heterogeneity amongst apamin sensitive K^+ channels, and suggest that UCL 1530 and related compounds should be useful tools for investigating this diversity.

Supported by the Wellcome Trust and the DGICYT.

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132P ADENOSINE A_1 AGONIST N6-CYCLOHEXYLADENOSINE AUGMENTS VASORELAXATION TO LEVCROMAKALIM IN ISOLATED PERFUSED RABBIT EAR

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We have recently shown that vasorelaxation elicited by levcromakalim in the isolated perfused rabbit ear is augmented by hypoxia (Randall & Griffith, 1993). Recent reports indicate that adenosine receptors may be coupled to potassium channel opener (KCO) sensitive potassium channels (Kirsch *et al.*, 1990) and we have now investigated the effects of N^6-cyclohexyladenosine (CHA), a selective adenosine A_1 agonist, on vasorelaxation to levcromakalim in the rabbit ear.

Rabbit ears were cannulated and perfused *in vitro* with oxygenated Holman's solution containing 10μM indomethacin. The preparations were preconstricted by addition of 5-hydroxytryptamine and histamine (both 1μM) and some preparations received CHA (1μM). Addition of CHA did not influence vascular perfusion pressure in the constricted preparations which was 129±10mmHg (mean±s.e.mean) before and 132±12mmHg after its addition. In all preparations the vasorelaxant properties of levcromakalim (10nM-10μM) were assessed. In the control situation the values of EC_{50} were 369±48nM and maximum relaxation (R_{max})=81.0±3.2% (n=7-15), while in the presence of CHA there was a significant ($P<0.01$) leftward shift in the concentration-response curve (EC_{50}= 194±54nM) and significant increase ($P<0.05$) in the maximum response (R_{max}= 89.9±2.7%, n=8). By contrast, vasorelaxation to sodium nitroprusside was not affected by addition of CHA (control EC_{50}=367±32nM and R_{max}= 84.9±4.7% and experimental EC_{50}=335±70nM and R_{max}=82.9±5.1%, both n=6).

The results show that vasorelaxation to levcromakalim is selectively augmented by the adenosine agonist CHA. Adenosine receptor activation may therefore potentiate KCO activity and raises the possibility that endogenous release of adenosine (eg in hypoxia) may influence KCO activity.

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133P 2-PHENYLADENOSINE IS AN ADENOSINE A_1 RECEPTOR BLOCKING DRUG

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We have previously shown (Gurden *et al.*, 1993) that the adenosine receptors mediating inhibition of the electrically-induced contractions of the guinea-pig ileum longitudinal muscle, the negative chronotropic response of the rat right atrium and antilipolytic activity in rat isolated adipocyte are of the A_1 subtype. In the present study, we report the potency of 2-phenyladenosine (2-PA) as an adenosine A_1 receptor- blocking drug in the above preparations. In addition, the affinity of this compound at adenosine A_1 receptors in the dog isolated adipocyte and in rat brain membranes has been investigated.

The methods used to study adenosine receptor mediated responses in the guinea-pig ileum, rat right atrium and rat isolated adipocytes (Gurden *et al.*, 1993) and dog isolated adipocytes (Strong *et al.*, 1993) have been described previously. In each preparation, or for each batch of cells, a cumulative concentration-effect curve for 5'-N-ethylcarboxamidoadenosine (NECA) was obtained in the absence and presence of up to three concentrations ($1x10^{-6}$ to $3x10^{-5}$ M) 2-PA, using a contact time of 30 min. The data were subjected to Schild analysis and antagonist potencies expressed as pA_2/pK_B values and the slopes of the regression analyses were calculated where appropriate (Table 1)

Table 1:	Guinea-pig ileum	Rat right atrium		Rat adipocyte	Dog adipocyte
pA_2	6.66 (6.47-6.85)	6.68 (6.45-6.91)	pK_B	6.21 (6.05-6.36)	6.07 (5.89-6.24)
Slope	0.90 (0.83-1.00)	0.92 (0.86-0.97)			

Values in parentheses are 95 % confidence limits (C.L.) of 4 to 6 observations.

2-PA caused rightward displacements of the concentration-effect curves to NECA with similar pA_2 values and no apparent change in maximum responses in the guinea-pig ileum, rat right atrium and both adipocyte preparations. Furthermore, 2-PA, at concentrations of $1x10^{-5}$ M in the rat right atrium and $3x10^{-5}$ M in the guinea-pig ileum, did not antagonise responses to carbachol and noradrenaline respectively and there was no evidence that 2-PA exhibited agonist activity. In rat brain membranes competition for binding was determined using $1x10^{-9}$ M [^{3}H]DPCPX as the ligand. In these experiments a mean pKi value for 2-PA of 5.85 (95% C.L. 5.63-6.05, n=4) was determined, which was not affected by inclusion of the stable GTP analogue, GPPNHP ($1x10^{-3}$M). The functional results, together with the absence of a shift in the pKi value for 2-PA estimated in the presence of GTP in rat brain membranes, indicate that 2-PA has affinity for but little or no efficacy at adenosine A_1 receptors.

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134P CARDIOVASCULAR AND METABOLIC EFFECTS OF SDZ WAG 994, A SELECTIVE ADENOSINE A_1 RECEPTOR AGONIST, AFTER ACUTE AND CHRONIC DOSING IN THE RHESUS MONKEY

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Adenosine induces a wide variety of pharmacological effects by activation of P_1-purinoceptors of which four subtypes, designated A_1, A_{2A}, A_{2B} and A_3, have now been cloned (Abbracchio et al., 1993). Activation of the A_1 receptor subtype results in inhibition of lipolysis by adipocytes, suppression of renin release from the kidney and a spectrum of cardiovascular effects which vary markedly between species (Linden, 1991; Wagner et al., 1994). We report here the cardiovascular and metabolic effects in the Rhesus monkey of N^6-cyclohexyl-2'-O-methyladenosine (SDZ WAG 994) a new, orally active, highly selective agonist at the A_1 receptor subtype (Wagner et al., 1994).

Eight Rhesus monkeys (4 male) weighing 4.5-11.1 kg were trained to sit in restraining chairs and systolic blood pressure (SBP) and heart rate (HR) were measured plethysmographically from the arm. Blood was withdrawn from a cannulated saphenous vein for measurement of plasma renin activity (PRA), immunoreactive insulin (IRI), glucose, free fatty acids (FFA) and triglycerides (TG) by conventional techniques. SDZ WAG 994, 0.6 mg/kg, was administered twice daily by gavage for 11 days. Measurements were made prior to and following an intravenous glucose tolerance test (IVGTT) following acute (after a single dose) and repeated (after the last of 21 doses) drug administration.

Following a single oral dose of 0.6 mg/kg of SDZ WAG 994 (n=8), changes in SBP were minimal but there was a small (15 ± 4 b/min [mean ± s.e.mean] at 120 min) but sustained (> 5 h) fall in HR. PRA, and the plasma FFA and TG concentrations were decreased from baseline by 44 ± 6, 80 ± 3 and 57 ± 3 %, respectively at 60 min and remained close to these levels for the duration of the experiment (5 h). The plasma glucose concentrations measured 5, 15 and 30 min following the IVGTT were slightly greater in the SDZ WAG 994-treated animals than in the controls. Plasma IRI concentrations did not differ between vehicle and drug-treated animals. There were no significant differences in any of the parameters comparing the effects seen following the first (single) administration of SDZ WAG 994 and those following the last of 21 oral doses (n=4 each group).

Consistent with a selective adenosine A_1 receptor agonist action, SDZ WAG 994 induces sustained inhibitory effects on HR, the plasma FFA and TG levels and PRA. Tachyphylaxis did not occur following twice daily dosing for 11 days. The pharmacological profile of SDZ WAG 994 suggests therapeutic potential in certain cardiovascular and/or metabolic disease states.

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135P BINDING AND FUNCTIONAL PROPERTIES OF THE CLONED HUMAN ADENOSINE A_1 RECEPTOR

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Binding studies on adenosine receptors have been complicated by the presence of endogenous adenosine (see eg Prater *et al.*, 1992). This has necessitated the use of adenosine deaminase (ADA) and thus precluded the investigation of the binding properties of adenosine itself. We have investigated the binding and functional properties of the human A_1 receptor expressed in CHO cells, using a saponin pretreatment to permeabilize structures present in the membrane preparations. This pretreatment simplifies the binding properties and allows the detailed investigation of receptor-response coupling in membranes, even in the absence of ADA.

Binding studies were performed using cell membranes (80μg/ml) in assay buffer (NaCl 100mM, HEPES 20mM, $MgCl_2$ 10mM, pH 7.4) for 1h at 25°C. Cyclopentyl-1, 3-(dipropyl-2,3-^{3}H) xanthine ([^{3}H]DPCPX, an A_1 antagonist) in the presence of GTP(10^{-3}M) has an affinity of (2.4 ± 1.0) x 10^9M^{-1}. Competition curves between [^{3}H]DPCPX and cyclohexyladenosine (CHA, an A_1 agonist) in the presence and absence of ADA demonstrated that in assays using CHO cell membranes, substantial quantities of adenosine are present. The endogenous adenosine affects all measurements of binding, most notably decreasing the high affinity agonist binding component. These effects can only be partially removed by ADA. In addition, even in the presence of GTP, agonist/^{3}H-antagonist inhibition curves were complex.

The results suggested that in the membrane preparations there could be vesicular (or similar) structures in which access of ADA (to adenosine) and GTP (to G-proteins) on the extracellular and intracellular face of the receptors respectively is prevented. Binding artifacts resulting from the presence of these structures are substantially eliminated by adding 0.1% saponin to the initial homogenization buffer (20mM Tris, pH 7.4, 10mM EDTA) prior to membrane preparation. This method allows the characterization of the binding properties of the human A_1 receptor including, for the first time, the binding of adenosine itself (affinity constant for the low affinity state of the receptor, 3 x 10^5M^{-1}). In the presence of GTP, ^{3}H antagonist/agonist inhibition curves are now simple.

In saponin treated CHO membranes, CHA stimulates [^{35}S]GTPγ[S] binding to G-proteins (in the presence of 10μM GDP) with an EC_{50} of (0.16 ± 0.06)μM. CHA inhibits ^{3}H-DPCPX binding (in the presence of 1mM GTP) with a K_D of (0.8 ± 0.1)μM. In both the binding assay and this assay of function (measured under similar conditions), CHA has a comparable potency, indicating a lack of major amplification at this early stage of receptor-response coupling.

Fiona Cohen is the recipient of a Glaxo Scholarship.

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136P THE EFFECT OF METAL CATIONS ON [^{3}H]α,β-METHYLENE ATP BINDING TO RAT VAS DEFERENS MEMBRANES

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It has been shown previously that [^{3}H]α,β-methyleneATP ([^{3}H]α,β-meATP) labels high and low affinity sites in rat tissues and that the high affinity binding sites correspond to P_{2x} purinoceptors (Bo & Burnstock, 1990; Michel et al., 1993). In this study we describe the marked effects of di- and trivalent cations on [^{3}H]α,β-meATP binding to these sites in rat vas deferens. Assays were conducted as described previously (Michel et al., 1993) except that membranes were incubated with [^{3}H]α,β-meATP in 50mM Tris buffer (pH 7.4) for 40 min at 4°C with or without added cations.

Both $FeCl_3$ (pEC_{50}=6.4±0.1; n=3) and $AlCl_3$ (pEC_{50}=6.2±0.1; n=3) increased binding of 1 nM [^{3}H],αβ-meATP to vas deferens membranes (eg. Total binding (dpm); Control=946±127; Plus 30μM $FeCl_3$ = 18199±945; n=3). This effect was not specific, however, since non specific binding (NSB) was also markedly increased (e.g. NSB (dpm); Control=90±6, Plus 30μM $FeCl_3$=13970±155; n=3). In contrast, several divalent cations increased specific binding, while not affecting NSB. The following rank order of potency (pEC_{50} ;SEM<0.2; n=3-4) was obtained :- $ZnCl_2$ (5.4), $MnCl_2$ (4.5), $CaCl_2$ (4.1), $CoCl_2$ (4.1), $MgCl_2$ (3.7), $BaCl_2$ (3.1). The maximal % increase in binding (as % of control) varied from 187±22% for $MnCl_2$ to 315±19% for $BaCl_2$. The monovalent cation salts NaCl and KCl (0.1-30mM) did not affect binding. In saturation studies [^{3}H]α,β-meATP labelled 2 sites in the absence of added cations (see table). The radioligand affinity for site 1 was significantly increased by $CaCl_2$ and $ZnCl_2$. Interestingly, $MgCl_2$ and $ZnCl_2$ specifically increased the capacity of binding for site 2 (see table).

Addition	Site 1 pK_d	Site 1 B_{max}	Site 2 pK_d	Site 2 B_{max}
None	9.15 ± 0.10	5.08 ± 0.9	7.06 ± 0.13	38.0 ± 6.9
$CaCl_2$ (3mM)	9.56 ± 0.04*	8.64 ± 3.2	6.95 ± 0.13	56.1 ± 25.4
$MgCl_2$ (3mM)	9.39 ± 0.06	5.19 ± 1.6	6.99 ± 0.26	155.6 ± 46.3*
ZnCl2 (30μM)	9.46 ± 0.07*	7.11 ± 2.1	7.22 ± 0.05	153.6 ± 38.4*

Bmax is in pmoles.mg^{-1} protein
Data are mean±SEM (n=5)
* P<0.05 vs. no addition
2 way ANOVA followed by Dunnett's test

This study shows that cations exert marked effects on [^{3}H],αβ-meATP binding. While the effects of $FeCl_3$ and $AlCl_3$ appear to be mostly non-specific, $CaCl_2$ can selectively increase the affinity of the radioligand for the high affinity binding site. This enables the high affinity sites to be selectively labelled in the presence of 3mM $CaCl_2$ and we have previously shown that these sites represent P_{2x} purinoceptors (Michel et al., 1993). The binding of [^{3}H]α,β-meATP to its low affinity sites is markedly increased by $MgCl_2$ and we are currently characterising these sites by performing assays in the presence of 3mM $MgCl_2$ and using a 100nM concentration of radioligand. The finding that $ZnCl_2$ could increase the affinity of [^{3}H]α,β-meATP binding for the high affinity site may provide additional evidence that this site is the functional P_2-purinoceptor since Cloues et al., (1993) have recently shown that similar concentrations of $ZnCl_2$ (1-10μM) enhance responses to ATP at a neuronal P_2 purinoceptor.

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137P A COMPARISON BETWEEN [3]α,β-METHYLENE ATP BINDING SITES AND FUNCTIONAL P_{2X} PURINOCEPTORS IN RAT VAS DEFERENS

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High and low affinity binding sites for [^{3}H]α,β-meATP have been reported in rat vas deferens (Bo *et al.*, 1992). In the present study we have determined dissociation pK_i estimates for a number of P_{2x} purinoceptor antagonists at the high affinity binding site in rat vas deferens and compared them to pK_b estimates from functional experiments on this preparation in order to determine whether the high affinity [^{3}H]α,β-meATP binding sites in rat vas deferens correspond to functional P_{2x} purinoceptors

In the binding studies, vas deferens membranes (from Sprague-Dawley rats 250-350g) were incubated in 250µl of 50mM Tris, 3mM $CaCl_2$ buffer (pH 7.4) for 40 min at 4°C in the presence of 1nM [^{3}H]α,β-meATP. Non specific binding was defined using 30µM α,β-meATP. Under these conditions >80% of the sites labelled display high affinity for α,β-meATP (Michel *et al.*, 1993). In some studies compounds were pre-incubated with membranes for 80 mins prior to addition of radioligand. Data presented (pK_i values and Hill slopes (nH)) are mean values±s.e.m of 4 separate experiments. Functional experiments were carried out using described methods (Khakh *et al.*, this meeting). After constructing a control concentration-effect curve, preparations were left to equilibrate with antagonists for one hour, except suramin which was left for 30 min (Mallard *et al.*, 1992). Second concentration-effect curves were constructed to α,β-meATP with an antagonist present throughout. pK_b estimates were determined from individual experiments at each antagonist concentration shown, and the values meaned (n = 4-5) to give an estimated pK_b for each antagonist (see table).

Antagonist	pK_b	pK_i	nH	pK_i*	nH*
†**PPADS** (1, 3 & 10µm)	7.0±0.1	5.3±0.1	0.6±0.04	5.9±0.3♦	0.5±0.04
†**CIBACRON BLUE** (100µM)	6.0±0.1	5.6±0.2	1.32±0.2	5.3±0.03	2.1±0.5
†**SURAMIN** (10, 30 & 100 µM)	5.5±0.1	5.5±0.1	0.7±0.04	5.4±0.1	0.8±0.1
TRYPAN BLUE (100µM)	4.9±0.4	4.3±0.1	0.9±0.1		
CONGO RED (100µM)	4.9±0.2	4.9±0.1	0.9±0.1		

♦Data fitted to two site model.
Site 1, pK_i =6.8±0.4; % of sites = 60±6.
Site 2, pK_i =4.5±0.5; % of sites = 40±6
† Antagonists were reversible on washout
* 80 min pre-incubation

With the exception of PPADS (Mclaren *et al.*, 1993), there was good agreement between the binding and functional affinity estimates. In the case of PPADS the affinity estimate from binding studies increased with a pre-incubation period of 80 min, suggesting it to be a slowly equilibrating antagonist. Furthermore, PPADS, similarly to α,β-meATP (Michel *et al.*, 1993), identified both high and low affinity [^{3}H]α,β-meATP binding sites. The pK_i at the high affinity site was similarly to the pK_b estimate of the compound in functional studies. These findings confirm the antagonist activity of suramin, reactive blue 2 and PPADS at P_{2x} purinoceptors using functional methods. The study has also identified trypan blue and congo red as two more putative antagonists which may add to understanding of structure activity relationships at P_{2x} purinoceptors. The close agreement between the pK_b and pK_i values for all the antagonists would suggest that the high affinity binding sites for [^{3}H]α,β-meATP are the same as, or similar to, functional P_{2x} purinoceptors in rat vas deferens.

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138P EVIDENCE THAT ADP ACTS AT P_{2Y}-PURINOCEPTORS AND NOT AT NUCLEOTIDE RECEPTORS ON BOVINE ARTERIAL ENDOTHELIAL CELLS

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Bovine aortic endothelial cells contain two types of phospholipase C linked receptors for ATP, and possibly ADP; P_{2Y}-purinoceptors and nucleotide receptors (Wilkinson et al, 1993). Here we show that stimulation of these receptors produces an endothelium dependent relaxation of vascular smooth muscle, and present evidence from both relaxant responses and inositol 1,4,5-trisphosphate (IP_3) production that ADP acts at the P_{2Y}-purinoceptor but not at the nucleotide receptor.

Bovine intercostal artery rings were mounted in Krebs solution in a 10 ml organ bath, under isometric conditions. Tissue was precontracted with 3 µM phenylephrine. Cumulative relaxant dose response curves to various nucleotides were constructed in the presence of 300 µM 8-sulphophenyltheophylline. Mass of IP_3 was determined in bovine aortic endothelial cells, by stimulation with nucleotides for 5s - 30s, with and without pretreatment with 100 ng ml^{-1} pertussis toxin for 16h. Data are mean ± s.e.mean.

Concentration response curves for relaxation of precontracted intercostal artery rings showed that the nucleotide receptor selective agonist UTP and the non-selective agonist ATP gave full relaxant responses. ADP, and the P_{2Y} selective agonist 2MeSATP, gave only partial (40-50%) relaxations at maximally effective concentrations. Curves to UTP were constructed in the presence and absence of supra-maximally effective concentrations (100 µM) of 2MeSATP or ADP. The pA_{50})-log EC_{50}) for the UTP control curve was 5.11 ± 0.09 (n = 18) while in the presence of 2MeSATP or ADP it was 5.03 ± 0.10 (n = 5) and 5.26 ± 0.08 (n = 5) respectively. We tested the relaxant responses for sensitivity to indomethacin (2.8 µM). The UTP response was unaffected by indomethacin, the responses to 2MeSATP and ADP were almost eliminated, while the response to ATP was shifted to the right: pA_{50} (n = 11) from 4.33 ± 0.08 to 3.95 ± 0.10 ($p < 0.05$). Measuring the pertussis toxin sensitivity of the IP_3 levels in cultured cells the % control (no pertussis treatment) stimulations were: ATP (300 µM), 64 ± 5; ADP (100 µM), 102 ± 18.7; 2MeSATP (30 µM), 90.2 ± 9.4; UTP (100 µM), 19.5 ± 2.1; (n = 3 or 4).

These results show that responses to ADP were not on the same receptors as those to UTP, in either cultured or in situ endothelial cells. Responses to ADP had the same characterisitics as 2MeSATP. Along with previously reported data (Purkiss et al, 1993) this suggests that while ATP interacts with both nucleotide receptors and P_{2Y}-purinoceptors, the effects of ADP are principally mediated by the P_{2Y}-purinoceptor and not the nucleotide receptor

This work was supported by the Wellcome Trust, MRC and Fisons plc.

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139P EXPRESSION AND LOCALIZATION OF THE A_{2A} ADENOSINE RECEPTOR IN THE HIPPOCAMPUS OF THE RAT

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High-affinity A_{2a} receptors, at which CGS 21680 is a potent agonist, are highly enriched in the striatum, but their excitatory role in the hippocampus has recently been described (Sebastião & Ribeiro, 1992). We now investigated the localization and expression of A_{2a} receptors in the hippocampus.

A_{2a} receptor binding was studied by autoradiography (Johansson *et al.*, 1993a), except that 30 nM [^{3}H]CGS 21680 and 12 weeks exposure were used. Thermocycling analysis was performed with two 25-mer oligonucleotide primers, specific for the dog A_{2a} receptor sequence (Schiffmann et al., 1990) and 50 ng cDNA reverse transcribed from poly(A)mRNA extracted by magneto-purification with streptavidin paramagnetic particles and a biotinylated oligo(dT) probe; after 35 cycles of 1 min at 92°C, 2 min at 55°C and 2 min at 72°C and a final extension of 5 min at 72°C, the products were analysed by agarose gel electrophoresis. *In situ* hybridization studies with a probe for A_{2a} receptor mRNA were essentially as described (Johansson *et al.*, 1993b).

Specific binding of [^{3}H]CGS 21680 was detected in the *strata moleculare, radiatum* and *oriens* of the *cornu Ammonis* with a greater density than in the sub- and supra-granular areas of the dentate gyrus. For example, specific binding to the *oriens* layer of CA_1 was 116 ± 8 fmol/mg grey matter as compared to 486 ± 15 fmol/mg grey matter in the anterior caudate-putamen (mean ± S.E.M. of 3 rats). Thermocycling analysis studies showed that A_{2a} receptor mRNA is expressed in the CA_1, CA_3 and dentate gyrus of the rat hippocampus, as well as in the striatum. *In situ* hybridization showed that the *stratum pyramidale* of the *cornu Ammonis* and the *stratum granulare* of the dentate gyrus were labelled with the probe for the A_{2a} receptor mRNA.

It is concluded that CGS 21680-sensitive A_{2a} receptors are not confined to the striatal system and that these receptors are expressed and localized in the rat hippocampus.

Work supported by Swedish MRC,Swedish Soc.Med.Res., Osterman's Found., 1987 Found.Stroke Res.,JNICT (STRD/SAU/292/92).

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140P THE ACTIVATION OF TONIC A_{2A} ADENOSINE RECEPTORS REGULATES NICOTINIC AUTOFACILITATION OF EVOKED [^{3}H]-ACh RELEASE FROM RAT MOTOR NERVE ENDINGS

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Adenosine has a neuromodulatory role at the rat neuromuscular junction by activating A_1-inhibitory and A_{2a}-excitatory presynaptic adenosine receptors (Correia-de-Sá *et al.*, 1991). It is known that acetylcholine (ACh) enhances its own release during repetitive nerve activity by stimulating facilitatory nicotinic autoreceptors at motor nerve endings. These nicotinic autoreceptors can be desensitized. It seemed, therefore, of interest to study whether adenosine could regulate ACh autofacilitation by interfering with the presynaptic nicotinic-induced desensitization.

The experiments were performed at 37°C on rat phrenic nerve-hemidiaphragm preparations loaded with [^{3}H]-choline (2.5 μCi/ml, 1 Hz, 40 min). [^{3}H]-acetylcholine ([^{3}H]-ACh) release was evoked by nerve stimulation using 750 pulses of supramaximal intensity and 40 μs duration applied at 5 Hz frequency. Two stimulation periods (S_1 and S_2) separated by a resting period of 24 min were used. The nicotinic agonist, 1,1-dimethyl-4-phenylpiperazinium (DMPP), was applied either 3 min or 15 min before S_2. When testing the ability of any drug to modify the effect of DMPP, the drugs were added to the preparations 15 min before each stimulation period.

The A_{2a}-adenosine receptor antagonist, 3,7-dimethyl-1-propargylxanthine (10 μM), greatly enhanced (60±5%, n=4), whereas the A_1-antagonist, 1,3-dipropyl-8-cyclopentylxanthine (2.5 nM), partially prevented (23±8%, n=3) the facilitatory (40±2%, n=4) effect of DMPP (1 μM, 3 min) on evoked [^{3}H]-ACh release. The A_{2a}-agonist, CGS 21680C (3 nM), but not the A_1-agonist, **R**-N^6-phenylisopropyladenosine (300 nM), partially blocked DMPP (1 μM) facilitation. Forskolin (FSK, 3 μM) mimicked the attenuation caused by CGS 21680C; inhibition of adenylate cyclase with MDL 12,330A (10 μM), potentiated (about 2.5 times) the facilitatory effect of DMPP. Prolonged exposure to a high concentration of DMPP (10 μM, 15 min) decreased evoked tritium outflow (20±4%, n=2). This nicotinic receptor desensitization was enhanced by CGS 21680C (3 nM)(41±5%, n=3) and FSK (3 μM)(42±3%, n=3), and was even reversed when inactivating endogenous adenosine with adenosine deaminase (0.5 U/ml).

The results suggest that activation of A_{2a}-adenosine receptors by endogenous adenosine, may regulate nerve evoked acetylcholine autofacilitation by favouring the desensitization of the presynaptic facilitatory nicotinic receptors, an effect probably related to the adenylate cyclase / cyclic AMP transducing system.

Aknowlegements: P.Correia-de-Sá is in receipt of a JNICT Ph.D studentship (BD/721/90-ID). The work performed in ICBAS was supported by a project Grant (PCMT/C/SAU/792/90) from JNICT.

P. Correia-de -Sá, A.M. Sebastião & J.A. Ribeiro (1991), *Br. J. Pharmacol.*, **103**, 1614-1620.

141P THE PHOTODYNAMIC ACTIONS OF δ-AMINOLAEVULINIC ACID ON RAT PANCREATOMA AR4-2J CELLS

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Photodynamic therapy (PDT) is an experimental treatment that selectively destroys tumour cells by an interaction between absorbed light and a retained photosensitising agent, generating highly reactive singlet oxygen, $^1O_2$. Protoporphyrin IX (PPix), a naturally occurring porphyrin in many cell types is one of the most effective endogenous photosensitizers. The biosynthesis of PPix can be enhanced by administration of exogenous δ-aminolaevulinic acid (ALA) especially in tumour cells (Malik & Lugaci, 1987), a pathway that we have demonstrated in the rat AR4 2J cell line.

AR4-2J cells from a rat pancreatoma cell line were routinely cultured to a subconfluent monolayer at 37°C and used at 80-90% confluence. Cells were resuspended in serum-free medium, seeded at a density of $4\text{-}5\times10^5$ cells per ml in 96-well plates, and allowed to attach in the presence of ALA prior to illumination on a modified Sigma T2203 illuminator at 4500 lux (λ>400nm). Cell survival was measured using an MTT/formazan conversion assay that measures intact mitochondrial activity in living cells.

Cell survival was found to decrease with increasing duration of illumination in cells treated with ALA (10μM). This photosensitivity was also light intensity dependent. After illumination for 8 min in the presence of ALA (10μM), exposure to light at 4500 lux resulted in a 40% reduction in cell survival to 62±1% of control values($P<0.05$); whereas, exposure to light at the higher intensity of 12000 lux led to a more than 80% reduction, ie., to 16±2% that of control values ($P<0.005$). Control values are defined by cell survival in the absence of both light and ALA. The dose-dependence of ALA-mediated phototoxicity was established with an EC_{50} of 12μM, at 4500 lux after 8 min. ALA,10μM and 100μM, did not generate singlet oxygen, whereas PPix (1μM) did, as determined by bilirubin photooxidation assay.

Porphobilinogen (PBG), an intermediary metabolite in porphyrin biosynthesis was detected in AR4 2J cells after 24 hours exposure to ALA. Control levels in the absence of ALA were 52.6±5.3nmol PBG/mg protein increasing to 65.8±8.6 nmol/mg protein (n=3) following exposure to ALA (10μM), and further to 103.4±10.3 nmol/mg protein ($P<0.01$, n=3) and 122.4±11.0nmol PBG/mg protein ($P<0.01$, n=3) after exposure to ALA, 20μM and 50μM, respectively. Using a simple absorbance method, the PPix content of AR4-2J cells was measured following exposure to different doses of ALA for 24 hours, by extraction in 0.1N NaOH and sonication. In untreated cells the PPix content was 2.5±1.1nmol/mg protein (n=6) increasing significantly to 5.0±1.6nmol/mg protein ($P<0.01$, n=6) with ALA (50μM) and further to 9.7±3.8nmol/mg protein ($P<0.001$, n=6) with ALA (100μM).

In summary, addition of exogenous ALA to AR4 2J cells will lead to PBG production and accumulation of photoactive PPix in the cells. Light activation of PPix may lead to the generation of $^1O_2$, a reactive species responsible for oxidative photodamage, in turn evoking time-, light intensity-, and ALA dose-dependent cytolysis of photosensitised cells.

SLR holds an MRC research studentship.

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142P CYCLOSPORIN A NEPHROTOXICITY: SITE SELECTIVE TOXICITY USING LLC-PK_1 AND MDCK CELLS

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Cyclosporin A (CsA) is a potent immunosupressive agent whose use is limited by nephrotoxicity (Kahan,1989). The aims of this study were to compare alterations in intracellular calcium and changes in cellular viability and cellular ultrastructure induced by CsA in cells from different areas of the nephron , in an attempt to elucidate a possible site selective action of the drug.

The established renal epithelial cell lines MDCK (distal tubular/collecting duct origin) and LLC-PK1 (proximal tubular origin) were used as the model system. Changes in cellular viability and ultrastructure were assessed using the neutral red cytotoxicity assay and transmission electron microscopy (TEM) respectively, following treatment from 4 to 24 hours with doses of CsA ranging from 4.2 nM to 83 μM. These doses approximate those reached in vivo in kidney tissue (Kahn *et al* ,1986).Calcium was measured using the calcium sensitive fluorescent probe Indo-1 AM in a B.D. Facstar Plus Flow Cytometer (Grynkiewicz *et al* ,1985).

A dose and time dependent loss of cellular viability was seen in both cell types for doses of CsA ranging from 4.2 μM to 83 μM.No significant loss in cellular viability was seen at doses of CsA less than 4.2 μM in either cell types ($p<0.05$). At the earlier time points of 4 and 6 hours the LLC-PK1 cells exhibited a greater loss of viability compared to that in the MDCK cells.Differences in sensitivities of LLC-PK1 and MDCK cells to CsA were also detected in ultrastructural changes.LLC-PK1 cells appeared to exhibit loss of microvilli, vacuolisation, mitochondrial and cytoplasmic damage and changes in cell shape following 4 and 6 hours treatment. Ultra structural changes were not as extensive in the MDCK cells.Resting $[Ca^{2+}]i$ was found to be 174 ± 50 nM in LLC-PK1 cells. CsA treatment for 24 hours increased the resting calcium by 138 ± 33, 175 ± 48 and 226 ± 67nM at doses of 4.2 nM,42 nM and 4.2 μM CsA respectively. Similar treatment did not cause elevations in $[Ca^{2+}]i$ in MDCK cells.

The ability of CsA to cause increases in $[Ca^{2+}]i$ at sub-lethal doses indicates that alterations in $[Ca^{2+}]i$ may play an important role in the mediation of toxicity by CsA .The greater susceptibility of LLC-PK1 cells to CsA induced damage indicates a possible site selective toxicity of CsA in the proximal tubular region of the kidney.

This work was supported by EOLAS and the E.C. BRIDGE Programme. Cyclosporin A was a gift from Sandoz Pharma.Ltd.

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143P COMPARATIVE PHARMACOLOGICAL PROFILE OF VINBLASTINE BINDING TO P-GLYCOPROTEIN OF THE LUNG CANCER CELL LINE H69/LX4 AND BREAST CANCER CELL LINE MCF-7-ADR

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P-Glycoprotein (P-gp) is a member of the ATP-Binding-Cassette (ABC) super family of transporters (Endicott and Ling, 1989). P-gp transports a wide range of cytotoxic drugs out of cells, including anthracyclines, vinca-alkaloids, epipodophyllotoxins and small peptides such as actinomycin D, rendering them multidrug resistant (MDR). It has recently been demonstrated that high affinity binding of [^{3}H]-vinblastine to P-glycoprotein does not require Mg^{2+}/ATP, allowing the binding assays to be performed in membrane fragments from the human lung cancer cell line H69/LX4 (Ferry et al, 1992). In this paper we explore the pharmacological profile of [^{3}H]-vinblastine binding in the human breast cancer cell line MCF-7-ADR, and compare it to that found in H69/LX4 membranes.

Partially purified surface membranes were prepared from H69/LX4 and MCF-7-Adr cell lines by homogenising ≈2 x 10^8 cells in ice cold Tris HCl pH 7.4 supplemented with 0.1 mM PMSF with an ultraturrax. Membranes were then isolated by centrifugation as previously described (Ferry et al, 1992). Binding assays were performed in 50 mM Tris HCl in a volume of 0.25 ml at 23° C allowing 60 min for equilibrium to be reached. For experiments with H69/LX4 or MCF-7-ADR membranes 10-20 µg and 25-35 µg of membrane protein were employed respectively.

Binding of [^{3}H]-vinblastine to membranes from MCF-7-ADR was of high affinity with a K_d of 24 ± 8 nM and B_{max} 25 ± 12 pmol/mg membrane protein (n=3). In membranes from the non P-gp expressing drug sensitive wild type cells (MCF-7 WT) no high affinity binding was found. A range of cytotoxics and MDR-reversing agents inhibited binding of [^{3}H]-vinblastine to P-gp of the MCF-7 Adr membranes. The K_i values (± SEM for n=3) for cytotoxics were; vincristine 603 ± 140 nM, etoposide 18 ± 5 µM, doxorubicin 31 ± 7 µM, and for MDR-reversing agents: cyclosporin A 18 ± 4 nM, niguldipine 15 ± 3 nM, azidopine 70 ± 14 nM, nicardipine 170 ± 50 nM, nifedipine 7.6 ± 2.8 µM, verapamil 450 ± 50 µM and quinidine 500 ± 180 µM. The correlation of K_i values for the P-gp of MCF-7-ADR and that of H69/LX4 (Ferry et al, 1992) for the 10 drugs is 0.996, with a slope of 1.15. The dissociation rate constant for [^{3}H]-vinblastine from P-gp of MCF-7-ADR membranes found by blocking the association reaction with 3 µM vinblastine is 0.021 ± 0.005 min^{-1} (n=9). The 1,4-dihydropyridines nifedipine (30 µM) and nicardipine (10 µM) accelerated [^{3}H]-vinblastine dissociation to 0.078 ± 0.043 min^{-1} and 0.20 ± 0.08 min^{-1} respectively. Cyclosporin A, doxorubicin and verapamil did not have a statistically significant effect. It is concluded that the P-gp of the breast cancer cell line MCF-7-ADR is very similar to the lung cancer line H69/LX4, with respect to affinities for cytotoxic drugs and MDR-reversing agents and the negative heterotropic allosteric effects of 1,4-dihydropyridines.

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144P CALCIUM-DEPENDENT PHOTODYNAMIC ACTION OF ALUMINIUM PHTHALOCYANINE TETRASULPHONATE ON RAT PANCREATIC EXOCRINE CELLS

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Phthalocyanines are efficient photosensitisers for the generation of singlet oxygen in experimental photodynamic therapy but the precise molecular and cellular mechanisms by which these compounds cause selective cytotoxicity, particularly on tumour cells, are not clearly understood. We have already reported that membrane-bound sulphonated aluminium phthalocyanine elicits amylase release from pancreatic acini upon light activation and that peak amylase release precedes peak prostaglandin E_2 production (Al-Laith, Matthews & Cui, 1993). These results suggested the possible involvement of G-proteins or PLA_2 activation either directly or via Ca^{2+} influx. The objective of the present investigation was to examine the role that Ca^{2+} may play in the release of amylase from normal rat pancreatic acinar cells and from tumour cells of the rat AR4-2J cell line when stimulated with light-activated aluminium phthalocyanine tetrasulphonate ($AlPcS_4$). Dispersed pancreatic acini, isolated by collagenase digestion, or cultured AR4-2J cells were loaded into tissue columns and perifused at 0.5 ml/min with oxygenated HEPES buffer at 37° C. Cells were illuminated for 10 min with a filtered quartz-halogen light source(λ >570 nm, 7000 lux) and amylase release was measured by a spectroscopic method.

Having demonstrated that there are no major changes in acinar cell structure after treatment by light-activated membrane bound phthalocyanine (Matthews & Cui, 1990), we now report that the photodynamic release of amylase is energy dependent. Preincubation of freshly-prepared acini cells with buffer containing antimycin A (10 µM), oligomycin (5 µM), and 2-deoxy-D-glucose (5 mM) before photoactivation of $AlPcS_4$, successfully blocked the release of amylase, from 7.14 ± 0.75 to 2.23 ± 0.49 times basal output (P< 0.001 n= 4-6). In contrast, the removal of extracellular Ca^{2+} had no effect on the release induced by light-activated $AlPcS_4$. However, depleting the intracellular Ca^{2+}, by treating the cells with BAPTA-AM (5 µM) before light-stimulation, decreased the photodynamic release of amylase to 2.97 ± 0.24 times basal output (P< 0.001, n=7). The photodynamic action of $AlPcS_4$ may therefore activate membrane receptors or G-proteins to initiate release of Ca^{2+} from intracellular stores and so trigger the release of amylase. This would explain why depleting the intracellular Ca^{2+} (but not extracellular Ca^{2+}) inhibits the release of amylase. We have also observed that amylase secretion is inhibited by the photodynamic action of $AlPcS_4$ on AR4-2J cells from 1.0 to 0.75 ± 0.12 times basal output (n=4). Removal of extracellular Ca^{2+} in this case produced a significant increase of amylase release to 1.44 ± 0.18 times basal output (P< 0.02, n=4). In sharp contrast BAPTA-AM (5 µM) had no effect on the inhibition of amylase release induced by light-activated $AlPcS_4$ in the AR4-2J cells which remained at 0.74 ± 0.07 relative to basal output (n=9).
This study has revealed further major differences between the photodynamic action of $AlPcS_4$ on normal acinar cells and those derived from a pancreatic carcinoma. It is concluded that changes in the control of intracellular Ca^{2+} are an important determinant of photodynamic drug action.

This work was supported by the Cancer Research Campaign.

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145P SK&F 96365 STIMULATES INOSITOL PHOSPHATE FORMATION IN HUMAN U373 MG ASTROCYTOMA CELLS

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SK&F 96365 is reported to be an inhibitor of receptor-mediated Ca^{2+} entry (Merritt *et al.*, 1990), although it also blocks voltage-dependent Ca^{2+} channels (Merritt *et al.*, 1990) and maitotoxin-induced Ca^{2+} entry (Soergel *et al.*, 1992). In C6 glioma cells phosphoinositide hydrolysis stimulated by maitotoxin, but not by endothelin-1, is also blocked by SK&F 96365 (Soergel *et al.*, 1992). We report here that in human U373 MG astrocytoma cells SK&F 96365 itself stimulates inositol phosphate formation.

U373 MG cells were grown in DMEM/F-12 nutrient mixture containing 10% fetal calf serum and 2 mM L-glutamine, and supplemented with penicillin and streptomycin. For the last 20-24 h growth the cells were labelled with 0.16 μM [^{3}H]-inositol in inositol-free medium containing 10 μM inositol. After dissociation cells were incubated for 15 min at 37°C in Krebs-Henseleit medium with 30 mM Li^+ before addition of stimulatory agents. Antagonists were added 15 min before SK&F 96365. Incubations, normally for 30 min, were terminated by addition of 10% perchloric acid and [^{3}H]-inositol phosphates ([^{3}H]-IP_x) separated by anion-exchange chromatography. [^{3}H]-IP_x formation in HeLa cells (S3 clone) and brain slices was measured as described previously (Arias-Montaño & Young, 1993).

SK&F 96365 stimulated a concentration-dependent accumulation of [^{3}H]-IP_1 (84 ± 5% of total [^{3}H]-IP_x at 100 μM SK&F 96365) in U373 MG cells in suspension (EC_{50} *circa* 20 μM and maximum response 5.5 ± 0.1 fold of basal, n=3) and in monolayers of the cells (maximum response 16 ± 1 fold of basal, n=3). Histamine (100 μM) produced an 11 ± 1 fold stimulation (n=31) in dissociated cells. SK&F 96365 also stimulated [^{3}H]-IP_1 formation in human HeLa cells (5.8 ± 0.4 fold of basal at 100 μM SK&F 96365, n=3), although it was less potent, but in slices of rat cerebral cortex and guinea-pig cerebellum 100 μM SK&F 96365 caused only a small stimulation of [^{3}H]-IP_1 accumulation (1.3 ± 0.1 and 1.4 ± 0.1 fold of basal, respectively, n=3). [^{3}H]-IP_3 accumulation induced by SK&F 96365 in U373 MG cells increased more slowly with time than that induced by 100 μM histamine. Accumulation of [^{3}H]-IP_1 was linear with time and was increased as the Ca^{2+} concentration of the medium was increased over the range 0.3 to 4.0 mM, but neither ionomycin nor A23187 (both 0.1 - 10 μM) stimulated [^{3}H]-IP_1 accumulation to the same extent as 100 μM SK&F 96365. KCl (10 - 50 mM) was without effect on [^{3}H]-IP_1 formation. Blockade of receptors known to mediate [^{3}H]-IP_1 accumulation in U373 MG cells, with methylatropine, prazosin, mepyramine and CP-96345 (all at 1 μM), did not alter the accumulation induced by 30 μM SK&F 96365. ω-Conotoxin (1 μM), nimodipine (1 μM) and verapamil (10 μM) were similarly without effect.

The mechanism by which SK&F 96365 induces [^{3}H]-IP accumulation in U373 MG cells and the nature of the dependence on extracellular Ca^{2+} remain unknown. Direct activation of a receptor coupled to phosphoinositidase C appears unlikely.

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146P EFFECTS OF LITHIUM ON AGONIST-STIMULATED CMP-PHOSPHATIDATE ACCUMULATION IN CEREBELLAR GRANULE CELLS, CHO-M3 CELLS AND SH-SY5Y NEUROBLASTOMA CELLS

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There is now considerable evidence to support the hypothesis that phosphoinositide cycle disruption plays a significant role in the anti-manic actions of lithium (Nahorski et al., 1991). Uncompetitive inhibition of inositol monophosphatase activity by lithium, prevents myo-inositol recycling and, if other sources of inositol cannot fully compensate, limits inositol phospholipid resynthesis. A sensitive index of cellular inositol depletion is the accumulation of CMP-phosphatidate (CMP-PA), the co-substrate with inositol for phosphatidylinositol synthesis. We have compared the effects of lithium on muscarinic receptor-stimulated CMP-PA accumulation in a number of cell-lines to establish under what conditions CMP-PA accumulation occurs.

Primary cultures of cerebellar granule cells, CHO cells transfected to express M3-muscarinic receptors and SH-SY5Y human neuroblastoma cells were cultured in 24-multiwell plates. The cellular CTP pool was labelled by incubating cells with [^{3}H]cytidine for 60 min at 37°C (Kennedy et al. 1990). After incubation in the presence of carbachol (CCH) + LiCl, reactions were terminated, and [^{3}H]CMP-PA was recovered, using the method described by Kennedy et al. (1990).

In cerebellar granule (CG) cells neither CCH (1 mM) nor LiCl (10 mM) significantly affected basal [^{3}H]CMP-PA accumulation. However co-addition of CCH+LiCl evoked a linear [^{3}H]CMP-PA accumulation which increased to 18 fold above basal values by 20 min after agonist challenge (basal, 1277 + 231; +CCH/Li, 22858 + 1877 d.p.m./well (n=3, independent triplicate experiments)). In CHO-M3 cells, 1 mM CCH caused a significant 5 fold increase in [^{3}H]CMP-PA, with the new steady-state being achieved by 10 min after agonist addition. In the presence of LiCl (10 mM), CCH evoked a substantially greater accumulation (basal, 445 + 95; +CCH/Li for 20 min, 30567 + 2215 d.p.m./well (n=3)). The increase in [^{3}H]CMP-PA over the initial 5 min was slow, consistent with depletion of cellular inositol being required for Lithium-sensitive CMP-PA accumulation in this cell-type. In both CG and CHO-M3 cells the increases in CCH-stimulated [^{3}H]CMP-PA (in the presence of 10 mM LiCl) were concentration dependent (pD_2 values 5.10 + 0.10 and 5.49 + 0.10 respectively). Likewise, LiCl concentration-dependently increased CCH-stimulated [^{3}H]CMP-PA accumulation (pD_2 values 2.57 + 0.10 and 2.67 + 0.05 respectively). Furthermore, in both cell-types [^{3}H]CMP-PA accumulation was prevented by pre-addition of myo-inositol (10 mM). In common with the CHO-M3 cell data, 1 mM CCH caused a 2-3 fold increase in steady-state [^{3}H]CMP-PA accumulation in SH-SY5Y cells, however neither the presence of Li, nor myo-inositol addition had any further effect on CCH-stimulated [^{3}H]CMP-PA in this cell-line.

These results suggest that the ability of lithium to affect agonist-stimulated [^{3}H]CMP-PA accumulation is not simply a function of stimulus strength, but is also crucially dependent upon the concentration of cellular inositol. These data also suggest that cerebellar granule cells in culture may provide a simple and suitable model system in which to further investigate the actions of lithium upon neuronal cell signalling.

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147P BRADYKININ AND CARBACHOL MOBILIZE INTRACELLULAR CALCIUM MORE POTENTLY THAN INOSITOL 1,4,5-TRISPHOSPHATE ACCUMULATION IN SH-SY5Y CELLS

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There is little doubt that inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) generated by phospholipase C (PLC) catalysed hydrolysis of phosphatidylinositol 4,5-bisphosphate mobilizes calcium from intracellular stores. Many cell surface receptors increase PLC activity thereby modulating intracellular calcium concentration ($[Ca^{2+}]_i$) although little is known of the relationship between $Ins(1,4,5)P_3$ formation and calcium mobilization in intact cells. A previous study in neutrophils (Thompson et al 1991) showed a 33-fold greater potency of fMet-Leu-Phe for calcium mobilization versus $Ins(1,4,5)P_3$ accumulation implying only 30-40% of maximal $Ins(1,4,5)P_3$ was required to fully mobilize calcium. It is unclear whether this is a general phenomenon or agonist and/or cell type dependent. This study examined $Ins(1,4,5)P_3$ and $[Ca^{2+}]_i$ elevations in adherent human neuroblastoma SH-SY5Y cells. While bradykinin provoked a rapid (10s) peak of $Ins(1,4,5)P_3$, determined by radioreceptor assay, which returned to basal levels by 60s, carbachol produced a peak followed by (>60s) a lower sustained agonist-dependent phase, although only peak responses will be considered here. The increase to maximal (10μM) bradykinin was only 2.32±0.27 fold (10) (mean±s.e.mean (n)) and had an EC_{50} (all $\log_{10}$ M) of -6.706±0.135 (8) (197nM) compared to a maximal (1mM) carbachol stimulation of 8.75±0.78 fold (11) with an EC_{50} of -4.626±0.072 (11) (23.6μM). In nominally calcium free conditions, (low $[Ca^{2+}]_e$: 2μM versus 2mM) basal $Ins(1,4,5)P_3$ levels were reduced (9.0±2.2 (6) versus 36.2±4.8 (11) pmols/mg protein) while the response to carbachol showed reductions of 10-20% at high carbachol conentrations, increasing to 80% at low concentrations but there was no difference in EC_{50}. There was also a marked reduction over the bradykinin dose-response curve with a reduction from 95.9±23.6 (4) to 26.4±4.8 (4) pmols/mg protein at maximal bradykinin. The pattern of alterations in $[Ca^{2+}]_i$, measured with fura-2, paralleled temporally the alterations in $Ins(1,4,5)P_3$ mass but had EC_{50}'s of -6.255±0.159 (0.55μM) for carbachol peak and -7.997±0.108 (16nM) for bradykinin. $[Ca^{2+}]_i$ responses to maximal carbachol and bradykinin showed 38% and 37% reductions respectively in low $[Ca^{2+}]_e$ and carbachol had an EC_{50} of -5.678±0.097 (2.1μM). These data suggest that under basal conditions and at low PLC activation $Ins(1,4,5)P_3$ accumulation was particularly sensitive to $[Ca^{2+}]_e$. This sensitivity declined at high activation levels possibly due to recruitment of calcium insensitive PLC isoforms or saturation of calcium enhancement by elevation of $[Ca^{2+}]_i$. Carbachol was more potent (43-and 12-fold at 2mM and 2uM $[Ca^{2+}]_e$) for $[Ca^{2+}]_i$ than $Ins(1,4,5)P_3$ elevation while bradykinin was12-fold more potent (2mM $[Ca^{2+}]_e$) and full $[Ca^{2+}]_i$ elevation occurred at a low % of maximal $Ins(1,4,5)P_3$ level indicating this is likely to be a general phenomenon. Also, for a given $Ins(1,4,5)P_3$ level, carbachol elevated $[Ca^{2+}]_i$ more than bradykinin, indicating involvement of other mechanisms in agonist-dependent $[Ca^{2+}]_i$ control.

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148P NITRIC OXIDE RELEASE INCREASES EXPRESSION OF C-FOS mRNA IN PRIMARY RAT STRIATAL CULTURES

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Nitric oxide (NO) has been identified as an intercellular messenger in the brain. Most of the actions of NO so far identified involve the stimulation of guanylate cyclase, with the consequent activation of cyclic GMP-dependent protein kinase (PKG). Recent evidence, from PC12 cells, suggests that NO can exert a long-term influence on cellular function by inducing the immediate-early gene c-fos (Peunova and Enkelpov,1993) although the involvement of PKG in this effect has yet to be shown. Nitric oxide synthase is abundant in the striatum, suggesting a major role for NO in modulating striatal activity. We have therefore investigated the ability of NO to stimulate expression of c-fos in primary striatal cultures. Striatal tissue from rat embryos (E17) was dissociated according to the method of Thayer et al (1986). Cells were seeded onto multiwell slides, and grown in Dulbeco's modified Eagles medium plus 10% horse serum for 10 days, with 20μM cytosine arabinoside added from days 4-7. Serum-free medium, supplemented with "ultroser" (Gibco), was used for a further two days, and then drugs were added. After 45 minutes, the cells were processed for in-situ hybridisation as described (Morris, 1989) using a ^{35}S-labelled 48mer oligonucleotide specific for c-fos mRNA (Wisden et al, 1990). The slides were exposed to film (1 week), and the hybridisation signal over each well quantified by computerised image analysis. Results were expressed as mean± sem (n = no. of determinations) in relative optical density units $x10^2$. Significance was assessed by ANOVA with post-hoc t-test. The c-fos hybridisation signal was low in cultures treated with vehicle (18.4 ±1.2, n=16) but was significantly increased (24.8 ±3.0, n=8, $p<0.05$) by treatment with 8-bromocyclicGMP (200μM). S-nitroso-n-acetyl-penicillamine (SNAP, 200μM), a spontaneous releaser of NO, also increased the c-fos signal (45.8 ± 4.2, n=8, $p<0.001$), and this effect was attenuated by coadministration of the PKG inhibitor KT5823 (2μM) to 31.0 ± 0.7, n=4, $p<0.001$ relative to vehicle, $p<0.05$ relative to SNAP alone. Conversely, coadministration of KT5720 (2μM), an inhibitor of cyclicAMP-dependent protein kinase (Kase et al, 1987), did not significantly affect the action of SNAP (42.0 ± 3.4, n=4, $p<0.001$ relative to vehicle). These results demonstrate that NO increases the expression of c-fos mRNA in rat striatum, and suggest that at least part of this action is mediated by stimulation of PKG.

We thank the Wellcome Trust for financial support, Prof.J.Garthwaite for the gift of SNAP, and Prof.A.Harvey and Dr.J.Pratt for access to the SIDR MCID system.

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149P INDUCTION OF C-FOS mRNA IN CULTURED RAT STRIATAL NEURONS BY DOPAMINE RECEPTOR STIMULATION

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The expression of a number of immediate early genes (IEGs) in rat striatum can be modulated by dopaminergic activity. Induction of the IEG c-fos has been observed in the striatum following treatment with amphetamine, apomorphine (Cole et al,1992), cocaine (Graybiel et al,1990), or the D1/D5 receptor agonist, SKF 38393 (Robertson et al, 1989). We have attempted to reproduce the dopaminergic induction of c-fos mRNA in primary cultures of rat striatal neurones, in order to characterise the receptor(s) involved. Striatal tissue from rat embryos (E17) was dissociated according to the method of Thayer et al (1986). The cells were seeded onto multiwell slides and grown in Dulbecco's modified Eagles medium with 10% horse serum for 10 days, with 20μM cytosine arabinoside added from days 4-7. Serum-free medium supplemented with "ultroser" (Gibco), was used for a further two days, and then drugs were added. After 45 minutes the cells were processed for in-situ hybridisation as described (Morris, 1989) using a ^{35}S-labelled 48mer oligonucleotide specific for c-fos mRNA (Wisden et al, 1988). The slides were exposed to film (1week), and the hybridisation signal over each well then quantified by computerised image analysis. Results were expressed as mean ± sem (n = no. of determinations) in relative optical density units x 10^2. Significance was assessed by ANOVA with post-hoc t-test.The hybridisation signal for c-fos mRNA was low in cultures treated with vehicle (12.36 ± 0.99, n = 31), but was significantly increased by treatment with dopamine (5μM), (27.36 ± 3.29, n = 11, $p<0.01$), and similarly with SKF 38393 (1μM), (24.20 ± 2.00, n = 20, $p<0.01$). Coadministration of the D1/D5 receptor antagonist, SCH 23390 (1μM) attenuated the induction of c-fos in response to 5μM dopamine (11.67 ± 0.88, n = 3, $p<0.05$ relative to dopamine alone), and in response to 1μM SKF 38393 (16.64 ± 1.27, n = 11, $p<0.05$, relative to SKF 38393 alone). The effect of SKF 38393 was also reduced by H7(50μM) to 5.80 ± 1.44, n = 10, $p<0.01$. These results show that the expression of c-fos mRNA in cultured rat striatal neurones can be stimulated by dopamine via a D1-like receptor.

We thank the Wellcome Trust for support, and Prof.A.Harvey and Dr.J.Pratt for the use of the SIDR MCID system.

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150P CHRONIC L-DOPA ADMINISTRATION INDUCES DYSKINESIAS IN THE MPTP-TREATED MARMOSET

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Dyskinesias occur in the majority of parkinsonian patients who are chronically treated with L-DOPA, along with a loss of response and motor fluctuations, which represent the other long-term complications of therapy with this agent (Nutt, 1990). L-DOPA induced dyskinesias have been reported in several non-human primate species after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity (Bédard et al., 1986). The common marmoset (*Callithrix jacchus*) develops a chronic parkinsonian syndrome after MPTP administration (Jenner et al., 1984) and we now submit the first report of dyskinesias occuring after chronic L-DOPA exposure in this species.

Marmosets (n= 4) rendered chronically parkinsonian by administration of MPTP (2 mg/kg daily for five days) were treated with L-DOPA (12.5 mg/kg p.o.twice daily) and carbidopa (12.5 mg/kg p.o. twice daily 30 min prior to L-DOPA) for three weeks. The animals were monitored by skilled observers and dyskinetic activity was graded on a semi-quantitative dyskinesia scoring system. Automated locomotor activity measurements were generated employing an infrared computer-linked system. After 7-10 days of dosing the animals began to display choreic and choreoathetoid dyskinetic movements and stereotypies which were most commonly seen in the upper extremities. Dystonic movements, consisting of arm retraction/adduction and leg flexion/extension movements, as well as orofacial dyskinesias, myoclonus, complex stereotypic behaviours and apparent akathisia were also seen. There was a wide range of severity of dyskinesias in the animals, with the most severely parkinsonian animals displaying the most dyskinetic movements. Each animal displayed an idiosyncratic pattern of dyskinetic activity, which was highly reproducible with repeated L-DOPA administration.

These L-DOPA primed animals also received other D-2 and mixed D-1 and D-2 agonist compounds with antiparkinsonian activity in both effective and high doses (relative to their actions on MPTP-induced akinesia), with three to four weeks between the introduction of each drug. Quinpirole (0.1 mg/kg and 0.3 mg/kg i.p.), apomorphine (0.075 mg/kg and 0.15 mg/kg s.c.), and bromocriptine (0.25 mg/kg and 1.0 mg/kg s.c.) all produced dyskinesias in a dose-dependent fashion which were identical in character and distribution to those seen after L-DOPA administration.

These observations increase the number of known primate species in which chronic L-DOPA administration will induce dyskinesias after MPTP-induced striatal denervation. The MPTP-treated common marmoset provides a convenient tool with which to study the factors responsible for dyskinesias in parkinsonian patients undergoing L-DOPA therapy and may lead to effective strategies in alleviating this common and troublesome side-effect of chronic dopamine replacement therapy.

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151P PHARMACOLOGICAL CHARACTERISATION OF BEHAVIOURAL STIMULATION TO SK&F 83959, A PARADOXICAL 'D_1-LIKE AGONIST' THAT *INHIBITS* DOPAMINE-SENSITIVE ADENYLYL CYCLASE

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Dopamine [DA] receptors appear to show greater heterogeneity than envisaged initially within the D-1/D-2 schema, in terms of both linkage to second messenger / transduction systems other than adenylyl cyclase [AC] and of molecular biological diversity (Waddington, 1993). The benzazepine SK&F 83959 has been identified as inhibiting the stimulation of AC induced by DA, i.e. to act as a D-1 antagonist, yet paradoxically exerting certain D-1 agonist effects on behaviour (Rogers et al, 1990; Arnt et al, 1992). We have recently described this compound to induce both intense grooming and vacuous chewing (Downes & Waddington, 1993), and have now characterised these responses further.

Using previously described procedures (Downes & Waddington,1993), rats [n=8 per group] were challenged s.c. with vehicle or 0.01-1.25 mg/kg SK&F 83959, or with 0.05 mg/kg SK&F 83959 following pretreatment with vehicle or the selective D-1 antagonists SCH 23390 and BW 737C, or the selective D-2 antagonist YM 09151-2 (Daly & Waddington, 1992), and resultant behavioural responses assessed. SK&F 83959 at 0.01-0.05 mg/kg induced intense grooming [$P<0.001$] and vacuous chewing [$P<0.001$]; these responses tended to decline at higher doses with the emergence of sniffing [$P<0.001$], rearing [$P<0.05$] and chewing [$P<0.001$]. Intense grooming was readily blocked by 0.01-1.0 mg/kg SCH 23390 [-100%, $P<0.001$] and by 0.04-5.0 mg/kg BW 737C [-85%, $P<0.001$], and also by 0.005-0.5 mg/kg YM 09151-2 [-77%, $P<0.01$]. Conversely, vacuous chewing was not blocked by SCH 23390, which induced vacuous chewing when given alone [+975% over vehicle, $P<0.01$], or by BW 737C, and was enhanced [+84%, $P<0.01$] by YM 09151-2.

Intense grooming was induced by SK&F 83959 and blocked by each of two chemically distinct selective D-1 antagonists; thus, SK&F 83959 appears to stimulate a 'D-1-like' receptor unlinked to AC but participating in cooperative D-1:D-2 interactions that regulate such typical DAergic behaviours (Waddington, 1993); conversely, the failure of these antagonists to block vacuous chewing suggests either an action at a distinct 'D-1-like' site , at which SCH 23390 also shows agonist activity and which participates in oppositional D-1:D-2 interactions that regulate such atypical behaviours , or the involvement of non-DAergic mechanisms.

This work was supported by the Royal College of Surgeons in Ireland. We thank Schering, SmithKline Beecham, Wellcome and Yamanouchi for gifts of drugs.

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152P INVESTIGATION OF PUTATIVE D_1:D_2 DOPAMINE RECEPTOR INTERACTIONS IN POST-MORTEM HUMAN BRAIN

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Evidence for dopamine (DA) D_1:D_2 receptor interactions is now substantial (Waddington *et al.*, 1993). This evidence has derived principally from behavioural studies, where both synergistic and oppositional D_1:D_2 interactions have been observed. At the cellular level, Seeman *et al.* (1989) have reported a link between D_1 and D_2 receptors by the use of the selective D_1 antagonist SCH 23390, which prevented the DA and quinpirole (D_1 agonist)-induced decrease in density of human D_2 receptors labelled with [^{3}H]-raclopride. Similarly, the D_2 antagonist eticlopride prevented the DA-induced decrease in [^{3}H]-SCH 23390 binding to D_1 receptors. In both instances, these effects were similar to the action of the GTP analogue Gpp(NH)p, suggesting that the link between D_1 and D_2 receptors was mediated by G-proteins. In the present study, [^{3}H]-SCH 23390 binding was determined in post-mortem putamen, caudate and nucleus accumbens homogenates and these phenomena examined.

Human post-mortem putamen, caudate and nucleus accumbens (mean post mortem interval 35.2 ± 15.5 h (n=6)) were prepared as described previously (O'Boyle & Waddington, 1987). Homogenates were pre-treated with 200nM eticlopride for 1 h at room temperature. Saturation experiments were performed using 0.06- 4.0nM [^{3}H]-SCH 23390 in the presence and absence of 400nM DA at 37°C for 30 min.

In agreement with the results of Seeman *et al.* (1989) we observed a concentration-dependent decrease in the apparent maximum number of [^{3}H]-SCH 23390 binding sites (Bmax) in the presence of 100nM - 2μM DA and that pre-treatment with 200nM eticlopride (100nM final concentration) affected neither Bmax nor Kd. However, we failed to observe any reversal of the effect of 400nM DA after pre-treatment with eticlopride. Bmax and Kd values are presented in Table 1. Similar results were obtained in caudate.

Table 1. Binding parameters for [^{3}H]-SCH 23390 in homogenized human post-mortem putamen and nucleus accumbens

		Kd(nM)	Bmax(pmol/g)			Kd(nM)	Bmax(pmol/g)
PUTAMEN	Control	1.7±0.4	18.6±4.9	ACCUMBENS	Control	1.7±0.5	16.3±4.9
	400nM DA	2.0±0.9	14.2±4.3*		400nM DA	1.9±0.5	14.1±4.0#
	Eticlopride	1.6±0.3	17.6±3.7		Eticlopride	1.6±0.5	15.3±5.9
	400nM DA+ eticlopride	1.9±0.6	13.7±3.5*,†		400nM DA+ eticlopride	1.9±0.3	13.1±4.8*,§

Values are mean ± s.d. (n=4) *$p<0.05$, #$p=0.1$ relative to control; †$p<0.05$, §$p=0.06$ relative to eticlopride without DA

These studies were supported by the Health Research Board

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153P INHIBITION OF FOOD REINFORCED CONDITIONED BEHAVIOUR BY HALOPERIDOL AND TRAZODONE: AN *EX VIVO* STUDY MEASURING DOPAMINE RELEASE FROM STRIATUM

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Food reinforced operant conditioned behaviour ('positive conditioning'; PC) has been correlated with a *reduction* in the release of dopamine (DA) from corticostriatal slices of conditioned rat brains (Patel & Turnbull, 1991; 1993). This type of conditioning can also be *temporarily* extinguished by neuroleptic drugs such as haloperidol and the antidepressant trazodone. The question arose as to whether these drugs achieve their behavioural effects by acting on the modified DA release system in the conditioned brain to normalise it. This question was addressed in the following studies where DA release profiles were measured in corticostriatal slices from brains of rats whose conditioned response had been temporarily extinguished by the psychotropic drugs.

Male Wistar rats (aged 35 days), previously conditioned on a discriminated FI30 schedule for food reinforcement (Patel & Turnbull, 1991), were administered haloperidol ($0.1 mgkg^{-1}$), trazodone ($100 mgkg^{-1}$) or vehicle (0.9% NaCl) i.p. and were decapitated 1h later. Corticostriatal slices were taken immediately post-mortem, placed in a Perspex insert within a gold microelectrode chamber and superfused at 0.4ml/min with modified Krebs bicarbonate solution (pH 7.4, 37°C, gassed with $95\%O_2/5\%CO_2$). Electrical field stimulation was applied using biphasic rectangular pulses (10Hz, 2ms, 20mA). Two (2min) stimulations (S1 & S2) were applied 50 min apart and DA released into the superfusate was estimated by HPLC with electrochemical detection. The results for net efflux of DA (stimulated minus basal level) are summarised in Table 1 (mean ± s.e. mean; 'n' rats).

Table 1

Experimental Conditions	Net efflux of DA expressed as a % of total slice content	
	S1 Electrical stimulation	S2 Electrical stimulation
No conditioning + no drug	2.83 (0.50) n=13	2.95 (0.54) n=10
PC alone	1.38 (0.18) ** n=16	1.53 (0.27) * n=10
PC + vehicle	1.55 (0.55) n=6	1.80 (0.48) n=6
PC + haloperidol ($0.1 mg/kg^{-1}$)	1.33 (0.09) NS n=7	1.74 (0.17) NS n=7
PC + trazodone ($100 mg/kg^{-1}$)	3.57 (0.75) ††† n=6	4.06 (0.56)††† n=6

*P<0.05; **P<0.01 conditioned compared with naive; ††† P<0.001; NS = not significant compared with PC alone and with PC + vechicle (One way ANOVA).

These results are consistent with neurochemical changes associated with aversively conditioned behaviour in that food reinforced conditioned behaviour is correlated with a reduction in the stimulated release of DA within the striatum. This DA release is unchanged by haloperidol, but significantly enhanced by trazodone at doses which inhibit conditioning. As the conditioning process is always reflected in a reduced DA release pattern, the actions of haloperidol and trazodone which extinguish such conditioned behaviours do not appear to be achieved through a common action on striatal DA release.

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154P PARADOXICAL EFFECTS OF AN α_2-ADRENOCEPTOR AGONIST ON CORTICAL NORADRENALINE EFFLUX: COMPARISON IN HALOTHANE-ANAESTHETISED AND FREELY-MOVING RATS USING DIALYSIS

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Selective α_2-adrenoceptor agonists reduce general anaesthetic requirement (Bloor *et al.*,1992). To investigate a possible interaction between α_2-adrenoceptor function and general anaesthesia, we have used dialysis and compared the effects of the highly selective α_2-adrenoceptor agonist medetomidine (MED) on noradrenaline (NA) efflux in the frontal cortex of both halothane-anaesthetised (HA) and freely-moving (FM) rats.

Dialysis probes were implanted in the frontal cortex of male Sprague-Dawley rats (250-350g) under halothane anaesthesia and perfused with Ringers at 1.0µl/min. Experiments began 2 h later (HA) or 24 h after recovery from anaesthesia (FM). NA recovered in the dialysate was measured by HPLC-EC. To confirm a neuronal origin of NA, K^+ (60mM) and Ca^{2+} were added or removed from the perfusate respectively. MED was perfused in increasing concentrations (0.5µM, 5µM and 50µM), each for 1 hour, either alone, or in combination with the selective α_2-adrenoceptor antagonist atipamezole (ATIP).

TABLE 1 Electrolyte- and drug-induced changes in cortical NA efflux in dialysates of HA and FM rats.

		K^+ 60mM/20mins	Ca^{2+} removal	ATIP 0.5µM/3h	MED 0.5µM	MED 5µM	MED 50µM
HA	treatment	182 ± 8*	36 ± 10*	219 ± 10*	86 ± 12*	183 ± 30*	416 ± 69*
	ATIP + MED				170 ± 28+	244 ± 48	415 ± 105
FM	treatment	627 ± 144*#	42 ± 10*	164 ± 24*	64 ± 9*#	96 ± 10#	238 ± 45*#
	ATIP + MED				102 ± 31+	77 ± 16	87 ± 9+

Values are maximum changes in NA efflux (mean ± s.e.mean) expressed as percentages of basal efflux (n=4-5). *p<0.01: ANOVA, *post hoc* t-test (*cf.* basal). + p<0.01: ANOVA, *post hoc* t test (*cf.* MED treatment). # p<0.01: ANOVA, *post hoc* t test (*cf.* HA treatment).

There was no difference in the basal efflux of NA between HA (22.0±1.7 fmoles/20mins, n=35) and FM (19.2±1.6 fmoles/20mins, n=23) rats or its dependency on Ca^{2+} supply. Both MED-induced inhibition of NA efflux and depolarisation-induced NA efflux were attenuated by halothane. In HA (5µM and 50µM) and FM (50µM) rats, MED increased NA efflux. ATIP alone produced similar increases in NA efflux in both HA and FM rats and reversed the inhibitory effects of MED on NA efflux in both groups of rats. Only in FM rats did ATIP prevent the increase in NA efflux induced by MED.

The results suggest that there are differences in processes determining NA efflux in HA and FM rats. At certain concentrations the α_2-adrenoceptor agonist, MED, increased rather than reduced NA efflux but, in HA rats at least, this effect is unlikely to be mediated by α_2-adrenoceptors.

This work was supported by the MRC. The gift of medetomidine from Orion-Farmos Pharmaceuticals is greatly appreciated.

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155P DECREASED [^{3}H]AGONIST BINDING TO PRESYNAPTIC α_2-ADRENOCEPTORS AND ALTERED α_2-AUTORECEPTOR FUNCTION IN THE RAT BRAIN REVEALED BY SHORT-TERM 6-HYDROXYDOPAMINE LESIONING

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The release/synthesis of noradrenaline (NA) in the CNS is regulated by α_2-adrenoceptors which are thought to be inhibitory presynaptic autoreceptors on noradrenergic terminals (Starke et al., 1989). However, the presynaptic location of these central α_2-autoreceptors is still a matter of debate mainly because noradrenergic denervation with neurotoxins (e.g. 6-hydroxydopamine, 6-OHDA, for 14 or more days) does not decrease or even increase the binding of specific radioligands to brain membranes (e.g. see Groβ et al., 1985). Recently, short-term (3 days) DSP-4 lesioning reduced the density of brain α_2-adrenoceptors suggesting that presynaptic receptors can be measured by radioligand binding (Heal et al., 1993). The aim of this study was to quantitate the density and function of brain presynaptic α_2-autoreceptors after 6-OHDA lesioning. Male Sprague-Dawley rats (280-320 g) were injected at the level of the locus coeruleus with 6-OHDA (4 μg/each side, i.c.v.) to induce noradrenergic denervation of distal areas of the brain. Eight and 21 days later, the density of α_2-adrenoceptors was quantified using [^{3}H]clonidine binding (0.25 - 16 nM) defined with phentolamine (10 μM). The sensitivity of central α_2-autoreceptors (8 days post-lesion) was assessed by quantitating the effects of clonidine and idazoxan on the in vivo rate of tyrosine hydroxylation (synthesis of DOPA) (Pi & García-Sevilla, 1992). As expected, short-term (8 days) treatment with 6-OHDA depleted the content of brain NA (43-62%, $P<0.05$). This treatment also reduced the density of α_2-adrenoceptors in the cerebral cortex (42%, $P<0.001$, n=4), hippocampus (30%, $P<0.01$, n=4) and amygdala (28%, $P<0.05$, n=4) compared to that in sham operated rats ([^{3}H]clonidine, Bmax: 105±5, 106±5 and 143±13 fmol/mg protein, respectively, n=5). The decreases in the number of α_2-adrenoceptors were no longer apparent 21 days after 6-OHDA lesioning, except in the amygdala where the density of receptors remained decreased (35%, $P<0.05$, n=3). 6-OHDA treatments also showed a tendency to increase the affinity of [^{3}H]clonidine, specially in the hippocampus (Kd values decreased by 15-30%, $P<0.05$). In sham operated rats, clonidine (0.5 mg/kg i.p., 60 min) decreased (33-42%, $P<0.05$, n=5) and idazoxan (5mg/kg i.p., 60 min) increased (40-133%, $P<0.05$, n=5) the synthesis of DOPA in all three brain regions. After short-term (8 days) treatment with 6-OHDA, clonidine continued to decrease the synthesis of DOPA (21-52%, $P<0.05$, n=4), but idazoxan failed to increase it and even tended to decrease it (25-35%, n=4). These results were as expected for the involvement of an α_2-autoreceptor mechanism in the regulation of NA synthesis (i.e. tonic receptor activation by endogenous NA is a necessary condition only for the synthesis-enhancing effect of the antagonist idazoxan). Together, the data indicate that presynaptic α_2-adrenoceptors with functional features of α_2-autoreceptors can be measured by radioligand binding after noradrenergic denervation in the rat brain.

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Supported by FIS (Project 93/0641).

156P [^{3}H]NISOXETINE, A NOVEL LIGAND FOR NORADRENALINE (NA) UPTAKE SITES: CORRELATIONS WITH INHIBITION OF [^{3}H]NA UPTAKE AND LOSS OF NA NERVE TERMINALS AFTER DENERVATION

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Nisoxetine, a potent and selective noradrenaline (NA) uptake inhibitor, has been demonstrated, when tritiated, to be suitable as a radioligand for labelling NA uptake sites (Tejani-Butt et al., 1990; Tejani-Butt, 1992). In this study, we have compared the potency of several antidepressant drugs to inhibit [3H]nisoxetine binding and [3H]NA uptake in rat frontal cortex. In addition, we have examined the effects of progressive lesioning of noradrenergic neurones with the neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) on the number and affinity of [3H]nisoxetine binding sites.

Inhibition constants (Ki values) for [3H]nisoxetine binding (1 nM) and [3H]NA uptake (10 nM) were determined using rat frontal cortical membranes (7.5 mg wet weight/tube) and synaptosomes (2.5 mg wet weight/tube) defined by mazindol (1 μM) and desipramine (10 μM), respectively. For lesioning experiments, rats were given DSP-4 (10, 20, 50 and 100 mg/kg, ip) 30 min after zimeldine (10 mg/kg, ip). Three days later cortices were removed and divided. [3H]Nisoxetine binding was quantitated at 8 concentrations (0.1-12.8 nM). Loss of noradrenergic nerve terminals in the cortex was estimated by measurement of NA concentrations by h.p.l.c. with electrochemical detection (Heal et al., 1991).

Table 1 Ki values for inhibition of [3H]nisoxetine binding and [3H]NA uptake in rat frontal cortex

	Nisoxetine	NA		Nisoxetine	NA		Nisoxetine	NA
Desipramine	1.6 ± 0.2	1.7 ± 0.1	Maprotiline	20 ± 2	26 ± 2	Dothiepin	67 ± 7	70 ± 3
Protriptyline	6.2 ± 0.8	2.5 ± 0.5	Amitriptyline	23 ± 2	28 ± 1	Mianserin	130 ± 15	87 ± 10
Nomifensine	25 ± 0	8.0 ± 0.9	Imipramine	29 ± 2	29 ± 1	Fluoxetine	902 ± 74	320 ± 33
Doxepin	24 ± 3	18 ± 2	Clomipramine	52 ± 4	40 ± 2	Fluvoxamine	1761 ± 122	612 ± 67

Ki values (nM) ± s. e. mean (n = 3).

[3H]Nisoxetine binding to rat cortex was of high affinity. The binding data of both competition and saturation studies fitted to a single site binding model. [3H]Nisoxetine binding was potently inhibited by the selective NA uptake inhibitors desipramine and protriptyline (Table 1). In addition, a very good correlation was obtained between the potency of twelve antidepressant drugs to inhibit [3H]nisoxetine binding and [3H]NA uptake (Table 1; correlation coefficient 0.96, $P < 0.001$). DSP-4 (10, 20, 50 and 100 mg/kg, ip) dose-dependently depleted cortical NA concentrations by 51%, 73%, 100% and 100% ($P < 0.01$ for all doses vs saline control, n = 4 - 6; Dunnetts test) with no significant effects on 5-HT and dopamine. These depletions, which were used as a marker for loss of noradrenergic nerve terminals, were associated with a dose-dependent decrease in the number of [3H]nisoxetine binding sites by 20%, 49%, 86% and 97% ($P < 0.01$ for all doses except 10 mg/kg vs saline control, n = 4 - 6; Dunnetts test), with no change in binding affinity. Furthermore, a good correlation was obtained between cortical NA concentrations and the number of [3H]nisoxetine binding sites (correlation coefficient 0.87, $P < 0.001$). Taken together these data support the view that [3H]nisoxetine binds to a single population of homogeneous sites associated with the NA transporter complex.

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157P SENSITIZATION OF β-ADRENOCEPTOR DOWNREGULATION IN MOUSE CEREBRAL CORTEX IN RESPONSE TO A BRIEF SWIM TEST

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It has been proposed that cerebral cortical β-adrenoceptor downregulation underlies adaptation to repeated stress (Stone, 1979). Yet it is not known whether this receptor change influences behavioural or neurochemical effects of an acute stress challenge. For this reason, the behavioural and neurochemical responses to a brief swim have been compared in mice with previous experience of repeated mild stress (once-daily saline injection) or repeated treatment with a monoamine uptake blocker which causes cortical β-adrenoceptor downregulation in rats, at least.

Male CD1 mice (20-25g) were given sibutramine hydrochloride (3 mg/kg i.p.) or saline (10 ml/kg i.p.) once-daily for 10 days and killed 24 h after the final injection, together with an uninjected group ('naive' control). In a separate study, uninjected mice, and mice given saline or sibutramine pretreatment as described, were given a single 6 min swim 24h after the last injection: immobility was scored and mice were killed immediately afterwards, together with a 'naive' control group. The density (Bmax) and dissociation constant (Kd) of cortical β-adrenoceptors were estimated from the specific binding of (-)-4-(3-t-butylamino-2-hydroxypropoxy)-[5,7-^{3}H]-benzimidazol-2-one ([^{3}H]-CGP 12177; 0.01-3.6 nM), defined by (-)-isoprenaline HCl (100μM).

Table 1 Stress- and sibutramine-induced changes in cortical β-adrenoceptor density and swim-induced immobility

	β-adrenoceptor Bmax (pmol/g. protein)		β-adrenoceptor Bmax (pmol/g. protein)	Immobility (sec)
'Naive' control	94.0 ± 7.2	'Naive' control	93.8 ± 6.1	N/A
		Swim only	91.8 ± 5.2	183.9 ± 4.8
Saline	94.8 ± 5.2	Saline + swim	79.6 ± 4.7*	168.4 ± 4.0+
Sibutramine	75.4 ± 2.8**	Sibutramine + swim	71.9 ± 4.2**	165.4 ± 6.1++

All values are mean ± s.e. mean for 10-11 mice. *p<0.05, **p<0.01: ANOVA, *post hoc* t test (*cf* 'naive' control).
+p<0.05, ++p<0.01: ANOVA, *post hoc* t test (*cf* swim only group). No treatment altered binding affinity.

Only sibutramine pretreatment caused a lasting downregulation of β-adrenoceptors in animals taken directly from their home cage. Experience of the swim test did not affect β-adrenoceptors in uninjected mice but induced a rapid and significant reduction in both saline and sibutramine pretreated animals. However, sibutramine did not further enhance the effects of saline injection on swim-induced changes in β-adrenoceptors. These neurochemical changes were paralleled by behavioural changes: immobility in the swim test was reduced to the same extent in saline and sibutramine pretreated mice. It is concluded that previous experience of repeated mild stress facilitates β-adrenoceptor downregulation induced by an acute stress challenge. Mechanisms underlying this downregulation are unknown but they are not additive with those of a treatment known to cause β-adrenoceptor downregulation in unstressed animals.

S.D. is a SERC/CASE scholar, in collaboration with Boots Pharmaceuticals Research Department

Stone, E.A. (1979) *Res. Commun. in Psychol. Psychiat. & Behaviour* 4, 241-255

158P DIFFERENTIAL ANTAGONISM OF THE POSITIVE INOTROPIC EFFECTS OF ET-1 AND ET-3

C.J. Hatton, S.M. Stoggall & C. Wilson; Zeneca Pharmaceuticals, Cardiovascular Research, Alderley Park, Macclesfield, SK10 4TG.

The cardiac actions of endothelin (ET) include a positive inotropic effect (Ishikawa *et al.*, 1989). The aim of the present study was to investigate, by means of selective agonists and antagonists, the ET receptor subtypes involved in the inotropic response of the guinea-pig left atrium.

Guinea-pig left atria, mounted in 10ml baths containing Krebs' solution were paced at 3Hz (3V, 5ms) and isometric tension recorded. One cumulative agonist concentration-response curve was constructed in each preparation. Tissues were pre-incubated with antagonists for 30 min and control tissues run in parallel. Tension responses were expressed as a % of the maximum to isoprenaline (10μM).

Table 1. Effects of endothelin analogues

Agonist	E_{max} (% iso) ± s.e. mean	EC_{50} (nM) and 95% conf. limit	n
ET-1	23.8 ± 2.8	2.3 (3.4 - 1.6)	12
ET-2	29.1 ± 3.0	4.2 (7.4 - 2.4)	9
ET-3	26.2 ± 3.0	31.7 (39.1-25.6)	12

The potency of the ET isoforms is shown in Table 1. In addition, the ET_B selective agonists sarafotoxin S6c (Williams *et al.*, 1991) and BQ3020 (Ihara *et al.*, 1992a) were without effect up to 0.6μM. The selective ET_A antagonist BQ123 (Ihara *et al.*, 1992b), at concentrations of 3 and 10μM (n = 4-6), produced a rightward non-parallel shift of the ET-1 concentration response curve (concentration ratio for BQ123 10μM = 9.7 at EC_{40}). In contrast, 10μM BQ123 abolished the ET-3 response. Lowering the concentration of BQ123 to 0.01 and 0.03μM produced competitive antagonism of responses to ET-3 (concentration ratio for BQ123 0.03μM = 16.2 at EC_{40}).

The agonist potency order (ET-1 = ET-2 > ET-3 >> sarafotoxin S6c = BQ3020) suggests that the inotropic response is mediated by the ET_A receptor (Arai *et al.*, 1990). However, the greater potency of the antagonist BQ123 against responses to ET-3 than against those to ET-1, a result which has also been observed in rat aorta (Sumner *et al.*, 1992), may suggest that different receptors mediate the responses to these two isoforms.

Arai, H., Hori, S., Aramori, I., Ohkubo, H. & Nakanishi, S. (1990) *Nature* **348**, 730-732.
Ihara, M., Saeki, T., Fukuroda, T., Kimura, S., Ozaki, S., Patel, A., & Yano, M. (1992a) *Life Sci.* **51**, PL47-52.
Ihara, M., Noguchi, K., Saeki, T., Fukuroda, T., Tsuchida, S., Kimura, S., Fukami, T. *et al.* (1992b) *Life Sci.* **50**, 247-254.
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Sumner, M.J., Cannon, T.R., Mundin, J.W., White, D.G. & Watts, I.S. (1992) *Br. J. Pharmacol.* **107**, 858-860.
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159P THE EFFECTS OF THE ET_A RECEPTOR ANTAGONIST, BQ123, ON ISCHAEMIC ARRHYTHMIAS AND MYOCARDIAL INFARCT SIZE IN ANAESTHETIZED RATS

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Elevated plasma levels of ET-1 have been observed in patients with myocardial infarction (Stewart et al, 1991). We have shown previously that infusion of ET-1 exacerbates the arrhythmias associated with myocardial ischaemia (Garjani et al., 1993), suggesting a proarrhythmic role for endothelin. In an attempt to determine the role of the ET_A receptor-mediated events during ischaemia this study investigated the effects of the ET_A antagonist, BQ123 (Ihara et al., 1992), on arrhythmias and infarct size in anaesthetized rats. Male Sprague-Dawley rats were anaesthetized with sodium pentobarbitone (60 $mgkg^{-1}$ i.p.) and prepared for occlusion of the LAD coronary artery. For the arrhythmia studies, BQ123 (10, 50 and 100μgkg^{-1} min^{-1} i.v.) or saline was infused from 5 min before ligation and for the duration of the ischaemic period (30 min). Total counts of beats appearing as single ventricular ectopic beats (VEB's), salvos (couplets and triplets) and ventricular tachycardia (VT) were performed and the incidences of VT and ventricular fibrillation (VF) were noted for each group. In 6 of the saline-treated rats, and 6 rats given 50μgkg^{-1} min^{-1} BQ123, the period of ischaemia was followed by 3.5 hours reperfusion to determine the effects of BQ123 on myocardial infarct size. Drug/saline infusion was maintained throughout. Prior to sacrifice Evans blue solution was injected i.v. (0.5ml; 10% w/v) to delineate area at risk. The hearts were then removed, sliced and incubated in triphenyl tetrazoliim (1%) to identify infarcted tissue. Infarct size was expressed as a % of area at risk.

Table 1:Effects of BQ123 on ischaemic arrhythmias. $^{**}P<0.01$;$^{*}P<0.05$ compared to controls.

Group	n	Single VEB's	Salvo's	VT	Total	%VT	$\%VF_{Total}$	$\%VF_{Irrev}$
Control	17	262±41	68±9	1092±143	1423±112	100	59	24
BQ123								
10$\mu gkg^{-1}min^{-1}$	10	122±27*	29±10*	525±142*	677±159*	100	50	30
50$\mu gkg^{-1}min^{-1}$	10	287±51	55±16	656±270	998±301	100	70	40
100$\mu gkg^{-1}min^{-1}$	8	-	-	-	-	-	75	75*

None of the doses of BQ123 had any significant effects on heart rate or blood pressure. Table 1 shows a suppression of arrhythmias with the lowest dose of BQ123 tested but a loss of antiarrhythmic effect with increasing dose. With the highest dose there was an increased incidence of irreversible VF, thus no arrhythmia counts could be performed. It is unclear why the effect on arrhythmias is not dose-related but a U-shaped dose-response curve has recently been described for the antagonism of the pressor response to ET-1 by BQ123 (Bird et al, 1993). At 50μgkg^{-1} min^{-1} BQ123 infusion did not alter infarct size (49.8±1.5% of area at risk vs 45.7±3.1% in controls). While these results agree with other studies on infarct size (McMurdo et al., 1993) the findings from the arrhythmia studies suggest that experiments with lower doses of BQ123 are required.

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160P THE ENDOTHELIN RECEPTOR ANTAGONISTS BQ-123 AND PD 145065 REVERSE ESTABLISHED PRESSOR RESPONSES TO ENDOTHELIN-1 IN THE ANAESTHETISED RAT

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Endothelin-1 (ET-1) binds almost irreversibly to its receptors (Hirata et al., 1988), most probably explaining the prolonged nature of its effects *in vivo* and *in vitro* (de Nucci et al., 1988). We have previously shown *in vitro* that responses to ET-1 mediated by either ET_A or ET_B receptors may be reversed by the subsequent application of endothelin receptor antagonists (Allcock et al., 1993). Here, we have examined the effectiveness of the endothelin receptor antagonists BQ-123 (ET_A-selective, Ihara et al., 1992) and PD 145065 ($ET_{A/B}$-non-selective, Cody et al., 1993) in reversing the pressor effects of ET-1 in the anaesthetised rat.

Male Wistar rats (200-400 g) were anaesthetised with sodium thiopentone (120 mg kg^{-1}, i.p.). The trachea was cannulated to facilitate respiration, the right carotid artery to measure blood pressure and the left and right jugular veins for the administration of drugs. After surgery animals were allowed to stabilize for 30 min before receiving a 5 min infusion of hexamethonium (10 mg kg^{-1}, i.v.). Twenty minutes later ET-1 was infused i.v. at a rate of 10^{-11} mol kg^{-1} min^{-1} to give a sustained increase in mean arterial blood pressure (MABP). After 70 min BQ-123 (10^{-9} to 10^{-8} mol kg^{-1} min^{-1}) and/or PD 145065 (10^{-8} to 10^{-7} mol kg^{-1} min^{-1}) and/or vehicle was additionally infused into the animals. The antagonist/vehicle infusion was stopped after 60 min and blood pressure recorded for a further 60 min. The infusion of ET-1 was maintained throughout. All infusions were at a rate of 300 µl hr^{-1}.

Infusion of ET-1 increased the MABP from 93±1.5 mm Hg to 137±2.4 mm Hg after 70 min (n=29). While the ET-1 infusion was continued an additional infusion of BQ-123 caused a gradual and significant ($p<0.01$, two-way ANOVA) dose-dependent reduction in the pressor effect of ET-1. For instance, infusion of BQ-123 (10^{-9} mol kg^{-1} min^{-1}) reduced the MABP by 9.8±3.8 mm Hg after 60 min, and BQ-123 (10^{-8} mol kg^{-1} min^{-1}) by 29.3±4.3 mm Hg (n=4 for each). PD 145065 was much weaker such that at 10^{-8} mol kg^{-1} min^{-1} it had no significant effect and even at 10^{-7} mol kg^{-1} min^{-1} the elevation in MABP induced by ET-1 was only reduced by 11.8±8.0 mm Hg (n=5) after 60 min. Co-infusion of BQ-123 and PD 145065 caused smaller reductions in the established response to ET-1 than infusion of BQ-123 alone (two-way ANOVA). For instance, co-infusion of BQ-123 (10^{-8} mol kg^{-1} min^{-1}) and PD 145065 (10^{-8} mol kg^{-1} min^{-1}) for 60 min reduced the MABP by 14±4.2 mm Hg (n=4).

Thus, endothelin antagonists reverse the pressor effects of ET-1 *in vivo* although co-antagonism of ET_A and ET_B receptors or the co-administration of an ET_A receptor antagonist, BQ-123, and a mixed antagonist, PD 145065 produces less reversal than the application of an ET_A receptor-selective antagonist.

We thank Parke-Davis Pharmaceutical Research for a grant, and the Medicinal Chemistry Dept. for BQ-123 and PD 145065.

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161P A NOVEL NON-PEPTIDE, MYRICERONE CAFFEOYL ESTER (50-235) IS SELECTIVE FOR HUMAN CARDIOVASCULAR ET_A RECEPTORS AND INHIBITS ENDOTHELIN-1 RESPONSES IN ISOLATED HUMAN BLOOD VESSELS

J.J. Maguire, C.R. Bacon, M.G. Peter, M. Fujimoto* & A.P. Davenport. Clinical Pharmacology Unit, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QQ. * Shionogi Research Laboratories, Osaka, Japan.

Myricerone caffeoyl ester (50-235), an extract of bayberry bark, was the first non-peptide endothelin (ET) antagonist to be discovered (Fujimoto et al., 1992). In rat cardiac membranes 50-235 potently inhibited [^{125}I]ET-1 binding (K_i 78nM) but was much less effective an inhibitor of [^{125}I]ET-3 binding, indicating some selectivity of this compound for the ET_A receptor subtype. We have investigated the ability of 50-235 to displace bound [^{125}I]ET-1 from ET_A and ET_B receptors in human left ventricle and coronary artery and to antagonize ET-1-induced vasoconstriction in human isolated saphenous vein (SV), internal mammary artery (IMA) and coronary artery (CA).

For competition binding experiments 10μm cryostat cut sections of human left ventricle and media of coronary artery were incubated for 120 min. at room temperature in 50mM Hepes Buffer (5mM $MgCl_2$; pH 7.4) containing 0.1nM [^{125}I]ET-1 with or without concomitant addition of 50-235 (10^{-12}-10^{-3}M). Non-specific binding was defined by inclusion of 1μM ET-1 and protein determinations were made using a modified Lowry method. Binding parameters were calculated using the iterative curve fitting programme LIGAND (Munson and Rodbard, 1980).
Rings (2mm in width) of endothelium-denuded human SV, IMA and CA were mounted under appropriate resting tension in 25ml organ baths containing oxygenated Krebs-Henseleit solution (37°C). Cumulative dose response curves (DRC's) were constructed to ET-1 (10^{-10}-$7x10^{-7}$M) in the absence and presence of 50-235 (3-30μM). ET-1 responses were expressed as a % of the contraction elicited by 50mM KCl. pA_2 values for 50-235 were estimated from the equation : $pA_2 = \log_{10}$ (dose ratio-1 / concentration antagonist (M)).

50-235 inhibited [^{125}I]ET-1 binding in human left ventricle with ET_A K_D value of 150±60nM and ET_B K_D value of 172±42μM (ratio of ET_A to ET_B receptors, 55%:45%) and in coronary artery with ET_A K_D value of 6.8±2.9nM and ET_B K_D value of 3.4±1.2μM (ET_A:ET_B, 79%:21%) (n=3). These data suggest a 1000 fold selectivity of 50-235 for ET_A receptors compared to ET_B receptors in these tissues.
50-235 produced a parallel rightward displacement of the ET-1 DRC in SV, IMA and CA with estimated pA_2 values of 6.5±0.2, 5.9±0.1 and 6.2 ±0.1, respectively (n≥6). The maximum response to ET-1 was not affected indicating that 50-235 was acting competitively at the doses tested.

The results show that the carbon-nitrogen peptide bond is not crucial for endothelin antagonist activity and the discovery of a non-peptide selective for human ET_A receptors is an important step forward in the development of therapeutic agents for conditions in which endothelins may be of pathological relevance.

We are grateful to the Theatre staff at Papworth Hospital for permission to collect human tissue samples.

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162P VASCULAR EFFECTS OF ENDOTHELIN IN RAT CORPUS CAVERNOSUM

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Endothelin (ET) is a 21 residue peptide with both vasodilator and vasoconstrictor properties. In order to investigate a possible role of ET in penile erection, we studied the effect of ET-1 and ET-3 on corporal pressure (CP), and of ET-1 on CP response to parasympathetic nerve stimulation (Vardi et al, 1989), in the pithed rat. In this preparation, corporal pressure is maintained at a very low level (5.97 ± 0.92 mm Hg; SEM, n=8), and increase in CP is indicative of arterial vasodilation in the corpora (Vardi et al., 1989).

Rats were pithed under halothane anesthesia, and a fine (25 gauge) needle inserted into one corpus cavernosum for measurement of CP. Electrical stimulation (ES) was applied to the sacral part of the spinal cord. Injection of ET-1 or ET-3 (0.1-10 μg/kg i.v.) was followed by a dose-dependent, rapid rise in CP, systemic arterial blood pressure (BP), and CP/BP ratio. Subsequently, CP returned to control values while BP was still elevated. ET-1 and ET-3 had equivalent potencies in elevation of CP at low doses (0.1-3 μg/kg), but differed in response obtained to the highest dose tested (10 μg/kg). Whereas in the case of ET-3, CP/BP ratio increased up to 10 μg/kg, with ET-1, CP/BP ratio increased at doses up to 3 μg/kg, but declined at 10 μg/kg. The NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME; 20 mg/kg) but not the cyclo-oxygenase inhibitor indomethacin (10 mg/kg), antagonised the maximal rise in CP caused by submaximal corporal vasodilator doses of ET-1 (1 μg/kg; from 35.8 ± 4.2 to 19.1 ± 5.4 mmHg; $P<0.05$, n=5) and ET-3 (3 μg/kg; from 37.8 ± 7.1 to 9.9 ± 4.4 mmHg; $p<0.05$, n=5). The increase in CP/BP ratio caused by ES (2-20 Hz for 1 min), given after the direct corporal vasodilator phase, but during the pressor phase, of ET-1 administration, was not altered at 1 and 2 μg/kg, but was reduced at 5 μg/kg (to 30.9 ± 3.5 and 67.9 ± 8.1 % control at 2 and 20 Hz respectively; n=6).

The results indicate predominantly vasodilator effects of ET in corporal vasculature at low doses, with vasoconstrictor effects at higher doses, and are consistent with other data showing that ET may exert part of its vasodilator action by local release of NO. At present, it is not known whether ET is released by erectile stimulation, but if so, it could play a role in the ensuing corporal vasodilation.

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163P CHARACTERISTICS OF ENDOTHELIN RECEPTORS IN RAT MAIN BRANCH RENAL ARTERY

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Endothelins have been implicated in both cyclosporine- and ischaemia-induced renal failure, conditions characterised by reduced renal blood flow. Studies in rats suggest that both endothelin $(ET)_A$ and ET_B receptors mediate renal vasoconstrictor responses to ET-1 (Wellings *et al.*, 1993). This study aimed to characterise the endothelin receptors in rat main branch renal artery *in vitro.*

Isometric tension measurements were made from rings of rat (Sprague-Dawley) main branch renal artery, suspended (initial tension = 0.4g) between 100μm wires in Krebs-Henseleit solution (37°C) containing indomethacin (30μM) to inhibit prostanoid production. Endothelial integrity was assumed if acetylcholine (1μM) caused >70% relaxation of the tone induced by a submaximal (~EC_{80}) concentration of the thromboxane A_2 mimetic, U-46619. The endothelium was removed from some rings by rubbing with a stainless steel wire. Responses to ETs were assessed in the presence of the peptidase inhibitors, phosphoramidon (1μM) & bestatin (10μM).

In endothelium-denuded preparations, ET-1 and ET-3 (0.1nM-10μM) caused concentration-related contractions. ET-3 was ~12-fold less potent and produced approximately 80% of the maximum response to ET-1. Geometric mean (n=5-6) EC_{50} values of 9.8 and 120nM were obtained for ET-1 and ET-3, respectively. In contrast, the ET_B receptor-selective agonists, sarafotoxin S6c and [$Ala^{1,3,11,15}$]ET-1 (0.1nM-30μM) had no contractile effect (n=5), and did not cause any relaxation of endothelium-intact preparations, pre-contracted with an EC_{80} concentration of U-46619 (n=5). Pre-incubation (15 or 45 min) with the ET_A receptor antagonist, BQ123 (0.3-3μM) resulted in weak and inconsistent antagonism of contractile responses to ET-1 (Figure 1). Mean pA_2 estimates from single antagonist concentrations ranged from 5.8-6.3 (n=4-6). In contrast, BQ123 (0.3μM, 45 min incubation) was more potent and consistent as an antagonist of the effects of ET-3 (Figure 1), with an estimated pA_2 of 7.6±0.2 (n=5). A combination of BQ123 (3μM) and the putative ET_B receptor antagonist, IRL1038 (Urade *et al.*, 1992)(10μM, n=5) caused no greater antagonism of the contractile effects of ET-1 than that due to BQ123 alone.

Thus, contractile responses to ET-3 in rat main branch renal artery appear to be mediated via BQ123-sensitive ET_A receptors. However, while ET_A receptors also appear to contribute to the contractile effects of ET-1 in this tissue, these data raise the possibility that a novel BQ123-insensitive endothelin receptor may also be involved.

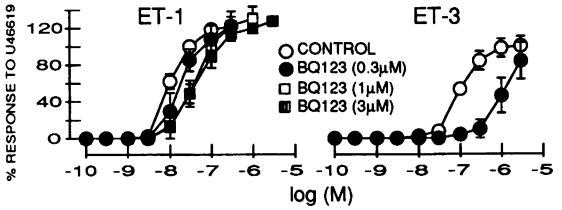


Figure 1. Effects of BQ123 on contractile responses to ET-1, and ET-3 (n=4-7).

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164P THE FUNCTIONAL EFFECTS OF IOTROLAN, AN ISO-OSMOLAR RADIOCONTRAST MEDIUM, ARE INHIBITED BY ENDOTHELIN ANTAGONISTS IN THE ISOLATED PERFUSED RAT KIDNEY

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The acute depression of renal function induced by radiocontrast media (RCM) has previously been demonstrated in the isolated perfused rat kidney(IPRK)(Brown *et al.* 1992). In this preparation, RCM produce a sustained fall in the glomerular filtration rate (GFR) and renal perfusate flow (RPF). RCM have been shown to increase endothelin release in tissue culture, in experimental animals and in man (Margulies *et al.*1992). In the present experiments, we have examined the involvement of endothelin in the acute renal response to iotrolan (Isovist 300), an iso-osmolar contrast medium in the IPRK using the endothelin antagonists Bosentan (Ro 47-0203),(4-tert-butyl-N-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)2,2′-d pyrimidine-4yl]-benzenesulphonamide) and BQ123, which is selective for the ET_A receptor.

Male Wistar rats (400-500g) were anaesthetised with thiopentone (125 $mgkg^{-1}$) and the right ureter and renal artery cannulated. Kidneys were perfused ex-vivo in closed circuit at constant pressure (100mmHg) with a modified oxygenated Krebs-Henseleit solution containing 6.7% albumin and 14mM mixed amino acids. RPF was continuously recorded while GFR was measured from the clearance of [^{14}C] inulin. Iotrolan was added to give a final perfusate concentration of 20mg iodine ml^{-1}. Either Bosentan or BQ123 were added 10 minutes prior to iotrolan at a final perfusate concentration of 10^{-6}M.

In control kidneys, basal levels of GFR and RPF were stable with time. Iotrolan produced a significant fall in both GFR (0.6 ± 0.01 to 0.45 ± 0.02 ml $min^{-1}g^{-1}$ $P<0.01$ n=6) and RPF (27.8 ± 1.4 to 20.8 ± 1.2 ml $min^{-1}g^{-1}$ $P<0.01$ n=6). In the presence of either Bosentan or BQ123, the depression of renal function caused by iotrolan was significantly reduced. Following Bosentan (10^{-6}M), iotrolan did not produce a significant fall in either GFR (0.61 ± 0.02 to 0.56 ± 0.02 ml $min^{-1}g^{-1}$ n=6) or RPF (28.8 ± 1.5 to 26.4 ± 1.1 ml $min^{-1}g^{-1}$ n=6). Furthermore, following BQ123 (10^{-6}M), iotrolan again did not produce a significant fall in either GFR (0.62 ± 0.08 to 0.60 ± 0.04 ml $min^{-1}g^{-1}$ n=6) or RPF (26.8 ± 1.67 to 25.0 ± 1.40 ml $min^{-1}g^{-1}$ n=6). No significant difference was observed between the inhibitory effects of Bosentan and BQ123, at the doses employed, on the renal response to iotrolan (ANOVA).The results indicate a role for endothelin, acting at the ET_A receptor, in mediating the acute depression of renal function caused by iotrolan in the IPRK.

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165P EFFECTS OF HYPOXIA ON THE RESPONSES TO ENDOTHELIN-1 IN THE ISOLATED GUINEA-PIG ILEUM

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Although first described as a vasoconstrictor, endothelin-1 (ET-1) has since been found to have contractile actions on most types of smooth muscle. Thus, Lin & Lee (1990) reported a biphasic response, relaxation followed by contraction, in the guinea-pig ileum in response to this peptide. We have previously reported that the vasoconstrictor effects of endothelins are potentiated by hypoxia (Douglas *et al.*, 1991) and that the vasodilator effects are maintained (Hiley *et al.*, 1993). Hence we here report a study intended to determine if the hypoxia-induced changes in the vasoconstrictor effects of ET-1 are common to all types of smooth muscle. The effects of hypoxia on the contractile responses to carbachol (CCh) were determined to give a comparison.

Dunkin-Hartley guinea-pigs (200-300g; Tucks, Rayleigh, Essex) were killed by a blow to the head and bled. The ileum was removed, the contents washed out with Krebs-Henseleit solution (composition in mM: NaCl 118, KCl 4·7, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25, $CaCl_2$ 2·5 and D-glucose 5·5), and 3 cm long segments were suspended in a 10 ml organ bath at 37°C. The Krebs-Henseleit solution in both the organ bath and the reservoir was bubbled with either 95% O_2:5% CO_2 (normoxia) or 95% N_2:5% CO_2 (hypoxia). The partial pressure of O_2 (PaO_2) in the solution was monitored with a Strathkelvin Instruments (Glasgow) model 781 meter and type 1302 O_2 electrode. The PaO_2 was 690±10 mmHg (n = 38) when the Krebs-Henseleit solution was bubbled with 95% O_2:5% CO_2, and 4.2±1.3 mmHg (n = 29) when bubbled with 95% N_2:5% CO_2. Changes in isometric tension were measured by a Grass FT-03 transducer coupled to a Grass model 79D polygraph. As maximal responses could not be obtained in all tissues due to desensitization, responses to ET-1 (Peptide Institute, Osaka, Japan) and CCh were expressed as percentages of the responses obtained in normoxia to 10 nM ET-1 and 1 μM CCh, respectively. CCh and carbonyl cyanide m-chlorophenyl-hydrazone (CCCP) were from Sigma.

Reducing the PaO_2 of the Krebs-Henseleit solution decreased the contractile potency of ET-1; there was a non-parallel shift in the log concentration/contraction curve with depression of the slope (responses to 3 nM & 30 nM in normoxia were 64.1±5.3%, n = 10, and 141±17%, n = 6, respectively, as compared to 28.9±5.1%, n = 3, and 51.2±3.4%, n = 3, in hypoxia). CCCP, which uncouples oxidative phosphorylation, gave a 10-fold parallel shift to the right of the log concentration/response curve for ET-1 in normoxia (responses to 30 nM and 300 nM were 61.7±8.2%, n = 3, and 131±6.3%, n = 3, respectively). Relaxation to ET-1 did not occur in hypoxia. In contrast, both hypoxia, and CCCP in normoxia, increased the responses to CCh. Responses to 0.1 μM and 10 μM were: normoxia, 40.3±2.8% (n = 10) and 169±18% (n = 9); hypoxia, 98.0±3.9% (n = 11) and 262±31% (n = 19); and CCCP in normoxia, 92.1±9.7% (n = 6) and 189±26% (n = 9).

Thus O_2 tension, and intracellular metabolic activity, differentially control the sensitivity of the ileum with respect to ET-1 and CCh but, unlike vascular tissue where endothelins are more potent in hypoxia, reducing the O_2 tension decreases the effectiveness of endothelin-1 as a contractor of intestinal smooth muscle.

This work was supported by a grant from the Marion Merrell Dow Research Institute, Strasbourg.

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166P SARAFOTOXIN 6c (S6c) DESENSITISATION OF THE CORONARY DILATOR RESPONSE TO ENDOTHELIN-1 (ET-1) IN PERFUSED RAT HEARTS

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ET-1 possesses coronary constrictor and dilator properties (Baydoun *et al.* 1989) which are thought to be mediated by ET_A and ET_B receptors respectively. The vasodilator component of the ET-1 response is lost following ischaemia/reperfusion (I/R) (Neubauer *et al.*, 1991) while the accompanying vasoconstriction is enhanced. It is known that vasodilatation (probably via the ET_B receptor) can be desensitised (Le Monnier de Gouville & Cavero, 1991). We have used the ET_B selective agonist S6c (Williams *et al.*, 1991) to exploit this desensitisation in assessing the role of the ET_B receptor in the actions of ET-1 in the rat heart.

Hearts from male Wistar rats were perfused at 10ml.min^{-1} with gassed (95%O_2:5%CO_2) Krebs Henseleit solution at 37°C, changes in coronary perfusion pressure isometric tension and heart rate being recorded. After 30min equilibration bolus doses of either S6c (100pmol) or an equal volume of vehicle were given at 10 minute intervals via an injection port, until desensitisation to S6C was apparent (3 doses). Following this, a bolus dose of ET-1 (30pmol) was injected. To test selectivity of the desensitisation, submaximal doses of bradykinin (10pmol) and sodium nitroprusside (10nmol) were injected following the desensitisation or the control period in separate preparations from those used for ET-1. The S6c dose was repeated to ensure continuing desensitisation. Results were analysed using Student's T-test or Mann-Whitney U-test and expressed as mean ± SEM.

S6c induced an initial vasodilatation of 29±3mmHg (n=10), which desensitised rapidly to a mean of 3±0.5 mmHg by the third dose ($P<0.001$). This desensitisation was maintained for 20min but was reduced at 30min. In control hearts, 30pmol ET-1 produced a biphasic response with an initial dilator component of 25±4mmHg (n=6) being followed by a constriction of 9±5mmHg. In S6c-treated hearts the vasodilatation to ET-1 was completely abolished in 6/6 hearts ($P<0.01$), and the accompanying constriction enhanced to a mean of 38±5mmHg (n=6, $P<0.01$). Changes in heart rate and developed tension were not significantly different between the control and desensitised groups. The vasodilator responses to bradykinin and sodium nitroprusside were unchanged by pretreatment with S6c ($P>0.05$, n=4 for both agents).

These experiments show a physiological antagonism between the coronary dilator and constrictor actions of ET-1. The loss of the dilator responses could account for the enhanced ET-1 - induced coronary constriction following I/R.

M.Thompson is a British Heart Foundation Student.

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167P HUMAN VASCULAR SMOOTH MUSCLE CELLS SECRETE BIG ET-1 AND MATURE ET BUT CONVERSION IS NOT BLOCKED BY PHOSPHORAMIDON

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There is evidence that animal vascular smooth muscle cells (VSMCs) are capable of secreting endothelin (ET) (Kanse SM, et al., 1991). ET-1 is a potent vasoconstrictor and a co-mitogen of VSMCs and therefore, may play a role in atherosclerosis, and post-balloon angioplasty. Our aim was to determine whether human VSMCs secrete mature ET and whether synthesis from its precursor, big ET-1, could be blocked by phosphoramidon, a putative endothelin converting enzyme inhibitor.

Following enzymatic dispersion, human umbilical vein smooth muscle cells (HUVSMCs) were grown to confluence in the absence of heparin to prevent growth of endothelial cells, which was confirmed by negative staining for von Willebrand factor (Dako) and positive staining for smooth muscle α-actin (Sigma). ET and big ET-1 were measured directly by radioimmunoassay over 12 hours with standard curves constructed using ET-1 and big ET-1 (Plumpton C, et al., 1992). Saturation binding was carried out using [^{125}I]ET-1 (Amersham) as previously described (Davenport AP, et al., 1993).

HUVSMCs secrete big ET and ET in a time-dependent manner (n=3, in triplicate). ET and big ET-1 were secreted in comparable amounts. The conversion of big ET to ET does not appear to be inhibited by 100 μM phosphoramidon (Student's t-test, $p<0.05$) (Table 1). Secretion of both peptides does not appear to be an artifact of enzymatic dispersion, nor specific to HUVSMCs, as big ET-1 and ET were both detected in the supernatants of explants from HUVSMCs and a range of adult human vessels (including coronary artery, thoracic aorta, pulmonary artery and saphenous vein).

Table 1.	CONTROL HUVSMC		PHOSPHORAMIDON HUVSMC	
time (hours)	irbig ET ± s.e.mean (pM)	irET ± s.e.mean (pM)	irbig ET ± s.e.mean (pM)	irET ± s.e.mean (pM)
4	392.9 ± 56.7	470.7 ± 59.0	493.0 ± 78.4	472.5 ± 91.4
8	750.5 ± 64.3	1023.4 ± 132.6	1024.5 ± 106.3	1013.3 ± 193.3
12	991.4 ± 145.6	1699.7 ± 182.4	1288.6 ± 132.6	1205.0 ± 186.2

Reverse transcription and polymerase chain amplification confirmed the presence of ET-1 mRNA in HUVSMCs (n=3). Saturation binding with [^{125}I]ET-1 revealed a single population of receptors on HUVSMCs with a K_D value of 256 - 454 pM and a B_{max} value of 33.3 ± 9.3 fmol / mg (n=6). Incubation of cultures with 100 μM phosphoramidon, or 100 μM pepstatin A for 48 hours did not alter the K_D values (phosphoramidon: 213 - 649 pM; pepstatin: 212 - 550 pM) or B_{max} values (phosphoramidon: 31.1 ± 12.7; pepstatin: 19.9 ± 7.8 fmol / mg) (n=3).

Thus, HUVSMCs secrete both big ET and ET, and express ET-1 mRNA. Conversion of big ET to ET-1 is not inhibited with 100 μM phosphoramidon. However, phosphoramidon has been shown to inhibit big ET conversion *in vitro* rat (Corder R, et al, 1993) and in HUVECs (Matsumura Y, et al, 1992). This suggests differences for big ET processing in human VSMCs and endothelial cells.

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168P EVIDENCE FOR TWO DISTINCT MECHANISMS INVOLVED IN MOTILIN/MACROLIDE CONTRACTILE ACTIVITY IN DIFFERENT SEGMENTS OF RABBIT INTESTINE *IN VITRO*

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Gastro-intestinal distress in patients treated with erythromycin is thought to be caused by motilin-like activity. We were interested to figure out whether the motilin-like activity is a characteristic feature of all macrolides (MA). For this purpose the effects of erythromycin (EM), clarithromycin (CM), roxithromycin (RO), oleandomycin (OM) and troleandomycin (TM) were investigated in isolated intestinal preparations of the rabbit and compared with that of motilin (MO) and carbachol (CCh). Duodenum (*DU*), jejunum (*JE*), ileum (*IL*) and sphincter of Oddi (*SO*), were investigated in order to take into account differences in segmental reaction pattern to MO. Shortening of longitudinal smooth muscle strips of the intestinal segments was recorded isotonically whereas contraction of *SO* was registered isometrically. Dose-response curves were established in a cumulative manner by increasing the concentration every 2min. Not more than 2 dose-response curves were obtained from one individual muscle.

segment /	parameter	motilin	carbachol	erythrom.	clarithrom.	roxithrom.	oleandom.	troleandom.
Duodenum	ED_{50}	0.001	0.25	1.2	1.2	1.4	22.9	19.4
	E_{max}	75.9±4.8	100	79.0±8.0	80.1±7.8	65.7±10.5	35.4±10.2	14.7±3.4
Ileum	ED_{50}	0.006	0.13	12.5	31.5	21.8	24.5	29.5
	E_{max}	17.3±3.1	100	29.9±4.6	22.4±2.9	23.4±3.4	29.5±7.9	22.9±4.3
	MIC (for Legionella pneumophila)			0.5-2	0.12-0.25	0.12-0.25	0.5-8	

Median effective dose (ED_{50} in μM), contractile activity (E_{max}: x±s.e.mean) expressed as a % of the individual maximum CCh effect (= 100%) with n = 5-7 animals. MIC = range of minimal inhibitory concentration (Bornstein et al., 1985).

From the motilin-like activity in *DU* there is no evidence for a dissociation of prokinetic and antimicrobial activity. Since EM-derivatives exist with strong prokinetic activity and lacking antimicrobial activity a dissociation of motilin-like and antimicrobial activity (Sunazuka et al., 1989) would have been expected. The response of MO and MA is strongly dependend upon the segment with the highest potency and efficacy in *DU*. EM, CM and RM proved to be as strong as MO itself in duodenal preparations whereas potency and efficacy were markedly reduced in *JE* (data not shown) and in ileal preparations values of the weak agonists OM and TM were reached. EM and MO stimulated *SO* to 30.1±3.2 (n = 7) and 25.0±3.0 (n = 13) respectively of the CCh-induced contractions whereas TM proved to be ineffective. The differences in efficacy of MO and EM, RM and CM between *DU* and *IL* were similar and neither the effects of MO nor that of MA were affected in presence of hexamethonium (10^{-6}M) and scopolamine (10^{-6}M) in these preparations indicating no contribution of neuronal tissue or m-cholinoceptors. The findings may be explained by the known decrease of the motilin receptor density from *DU* to *IL*. It's noteworthy to mention that no differences in efficacy could be detected in *IL* with MA and that the potency of MO and EM, RM and CM was 10 times lower than in *DU*. The missing differentiation between MO and MA in the ileum indicates at least two destinct mechanisms of contractile activity in this segment which needs further elucidation.

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169P CHARACTERISATION OF THE CONTRACTILE ACTION OF SOMATOSTATIN IN THE RAT ISOLATED DISTAL COLON

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In general, the neuropeptide somatostatin (SS_{14}) exerts inhibitory actions throughout the gastrointestinal tract (Gyr and Meier, 1993). We describe for the first time a contractile effect of somatostatin on the rat isolated distal colon, and in the present study we have attempted to characterise the somatostatin receptor mediating this action by comparing the relative potencies of several cyclic somatostatin analogues.

Segments (approx. 2cm) of whole distal colon were removed from male Sprague-Dawley rats (200-300g) and placed in organ baths containing modified Krebs solution (mM): NaCl (118), $NaHCO_3$ (25),KCl (4.7), $MgSO_4.7H_2O$ (0.6), KH_2PO_4 (1.2), D-glucose (11.1), $CaCl_2.6H_2O$ (1.3), at 37^0C and gassed with 95% O_2/ 5% CO_2. Changes in isometric tension were recorded from a resting tension of 1.5g. A non-cumulative concentration effect (c-e) curve to SS_{14} (1nM-1μM) was constructed on all preparations with a 15 min washout period between each dose. Sixty min later c-e curves to SS_{14} or a test agonist were repeated using a Latin square design. Thirty minute dosing intervals were used for experiments with BIM23027 (Raynor et al., 1993). Responses were expressed as % of the original maximal contractile response to SS_{14}. Equi-effective molar ratios (EMR) were measured from each c-e curve at the level causing 50% of the test agonist maximum, and corrected for spontaneous changes in agonist sensitivity.

TABLE: A comparison of the potencies of several SS analogues causing contractile responses in the isolated rat distal colon.

Analogue	BIM23027	[D-Trp^8]-SS_{14}	Octreotide	CGP23996	SS_{14}	Angiopeptin	SS_{28}
EC_{50} (nM) *	0.85(0.1-8)	2(0.2-17)	7(3-15)	15(2-11)	10(7-15)	18(5-70)	20(8-49)
EMR *	0.08(0.02-0.34)	0.14(0.02-0.94)	0.31(0.04-2.4)	0.58(0.19-1.8)	1	0.94(0.15-5.9)	1.58(0.5-4.5)
% Original SS_{14} max †	130±7	126±8	120±7	141±15	125±4	103±12	102±5

*Values expressed as geometric mean (95% confidence limit). †Values expressed as mean ± s.e.mean. n = at least 4 observations.

All compounds induced concentration-dependent contractile responses with similar maxima to SS_{14} (Table 1). BIM23027 and SS_{14}, [D-Trp^8]-SS_{14}, were the most potent agonists (approximately 12 and 7 times more potent than SS_{14} respectively). Following a 1hr incubation, the peptidase inhibitors phosphoramidon (1μM)) and amastatin (10μM) had no significant effect on the potency of SS_{14} or SS_{28} (EMR 1(0.02-6) and 3(1.2-8.7) respectively). The putative somatostatin receptor blocking drug cyclo 7-aminoheptanoyl-Phe-D-Trp-Lys-Thr[Bzl] (1μM, 1hr pretreatment) did not antagonise the effects of SS_{14} (EMR 0.8 (0.5-1.3). SS_{14} (1nM-1μM) evoked contractile responses were unaffected by atropine (1μM), but were inhibited by tetrodotoxin (1μM).

The most marked feature of the agonist profile observed (Table 1) was the high potency of BIM23027 and [D-Trp^8]-SS_{14} relative to SS_{14}. This agonist profile more closely resembles that obtained in radioligand binding studies on the recently cloned SSTR2 receptor than that of any of the other cloned somatostatin receptors subtypes (Raynor et al. 1993). The contractile action of somatostatin on the rat distal colon appears to involve activation of non-cholinergic nerves, however the precise site and mechanism involved remains to be elucidated.

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170P EFFECTS OF THE SOMATOSTATIN ANALOGUE, OCTREOTIDE, ON GASTRIC ACID SECRETION IN THE MOUSE ISOLATED STOMACH

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It is well known that somatostatin (SRIF-14) potently and almost completely inhibits gastric acid secretion stimulated by food or by muscarinic agonists in various species *in vivo*. Park et al. (1987) also described an inhibitory effect of SRIF-14 on isolated canine parietal cells *in vitro*; yet the potency and efficacy of SRIF-14 on isolated parietal cells appear to be quite weak, compared to the potent effects observed *in vivo*. Thus the question arises whether the inhibition of gastric acid secretion *in vivo* is a consequence of a direct effect on gastric parietal cells or whether it is an indirect effect. The standard model of the isolated, lumen-perfused mouse stomach (Black & Shankley, 1985) allows characterization of both the direct and indirect effects on gastric acid secretion and the determination of potency values which are not biased by the pharmacokinetics of the agents. Using isolated, lumen-perfused stomach preparations of the mouse, essentially as described by Black & Shankley (1985), we characterized the potency, efficacy and the mode of action of octreotide on gastric acid secretion *in vitro*.

Similar to the histamine H_2 receptor antagonist, cimetidine (up to 10^{-4} M), and the muscarine receptor antagonist, pirenzepine (up to 10^{-5} M), octreotide (up to 10^{-7} M) did not affect basal gastric acid secretion in this model. Only the H^+/K^+-ATPase inhibitor, omeprazole (10^{-4} M), proved to be effective under these circumstances, inhibiting basal gastric acid secretion by about 75 %. The muscarine receptor agonist, bethanechol, was a potent stimulant of gastric acid secretion [EC_{50}: 1.28×10^{-5} M; 5.84×10^{-6} M - 8.15×10^{-5} M (mean; 95 % confidence interval)]. The response was biphasic. The first phase was very rapid in onset, but only transient; in contrast, the second phase developed about 15 min after addition of bethanechol to the organ bath and was very long-lasting (> 120 min). Both effects could be completely inhibited by pirenzepine at 10^{-5} M. The histamine H_2 receptor antagonist, ranitidine (10^{-4} M), failed to inhibit the first phase of the bethanechol-induced acid secretion but abolished the second one. Octreotide (10^{-7} M) behaved similarly to ranitidine in that it did not affect the first phase of the bethanechol effect but completely suppressed the second one. The EC_{50} of octreotide for the inhibition of the second phase of the bethanechol-stimulated acid secretion was 6.31×10^{-9} M; 4.97×10^{-9} M - 8.12×10^{-9} M (mean; 95 % confidence interval). Histamine-stimulated (1×10^{-4} M, in the presence of isobutyl-methylxanthine, 3×10^{-5} M) gastric acid secretion, however, was not inhibited by octreotide at concentrations up to 10^{-7} M, and only marginally affected at higher concentrations. Ranitidine completely inhibited histamine-stimulated gastric acid secretion at 10^{-4} M.

These data suggest that bethanechol stimulates gastric acid secretion by two independent actions, one of which is mediated by histamine release from the gastric mucosa. We speculate that the first phase of the bethanechol response represents a direct stimulation of muscarinic receptors on murine parietal cells which is not or only very weakly inhibited by octreotide. The second phase of the bethanechol response is probably mediated by stimulation of histamine release from gastric enterochromaffin-like cells (Chuang et al., 1992) which is potently inhibited by octreotide.

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171P CHARACTERISATION OF THE RECEPTORS INVOLVED IN THE VASOCONSTRICTOR ACTION OF 5-HYDROXYTRYPTAMINE IN EQUINE DIGITAL VEIN

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The pathophysiology of the ischaemic disease, equine laminitis (EL), where the sensitive laminae of the horse's foot are starved of nutrient blood flow, is poorly understood. It is possible that arteriovenous shunting may be involved in the pathogenesis of EL. 5-HT is a potent vasoconstrictor of equine isolated digital blood vessels (Baxter et al., 1989) but the receptors mediating this vasoconstrictor effect have not been studied.

Rings of equine digital vein (EDV) were denuded of their endothelium and prepared for isometric tension recording as previously described (Bryant et al., 1992). Vessels (from 6 different horses) were incubated with benextramine (0.1 mM) and pargyline (0.5 mM) for 30 min and then washed with drug-free Krebs-Henseleit solution. Cumulative dose response curves (CRCs) were constructed to 5-HT (0.1 nM to 0.1 mM), α-methyl-5-hydroxytryptamine (α-me-5-HT; 0.1 nM to 0.1 mM), 5-carboxamidotryptamine (5-CT; 0.1 nM to 0.1 mM), tryptamine (TYP; 0.1 nM to 0.1 mM) 2-methyl-5-hydroxytryptamine, (2-me-5-HT; 10 nM to 0.1 mM) and 8-hydroxy-2-(N,N-dipropylamine) tetralin (8-OH-DPAT; 10 nM to 0.1 mM). CRCs to 5-HT and 5-CT were also obtained in the presence of ketanserin (KET; 30 nM) and methiothepin (MET; 10 nM).

All agonists produced dose-dependent increases in isometric tension. 5-CT produced a biphasic CRC with a plateau between 10 nM and 0.1 μM (estimated EC_{50} values of 1.16 ± 0.14 nM and 2.8 ± 0.26 μM and maximum responses (g) of 1.1 ± 0.03 and 2.6 ± 0.07). The order of potency (estimated EC_{50} values; maximum response (g)) of the remaining agonists was 5-HT (55.4 ± 3.8 nM; 5.3 ± 0.07), TYP (127.6 ± 9.6 nM; 3.7 ± 0.04), α-me-5-HT (131.4 ± 5.0 nM; 4.7 ± 0.03), 8-OH-DPAT (3.9 ± 0.2 μM; 2.4 ± 0.03) and 2-me-5-HT (6.1 ± 0.3 μM; 2.6 ± 0.05). In the presence of KET (30 nM), 5-HT produced a biphasic CRC with EC_{50} values of 10.8 ± 1.0 nM and 5.5 ± 0.7 μM. KET selectively inhibited the second part of the CRC to 5-CT producing a biphasic curve with a plateau between 10 nM and 10 μM. In the presence of MET (10 nM), 5-CT and 5-HT produced monophasic CRCs with EC_{50} values (nM) of 63.1 ± 4.9 and 60.6 ± 4.9 and maximum responses (g) of 0.7 ± 0.01 and 2.49 ± 0.03 respectively ($p<0.05$ when compared with the maximum responses obtained in the absence of MET using an unpaired Student's *t*-test).

The high potency of 5-CT, the resistance of part of the CRCs to 5-CT and 5-HT to inhibition by KET and the sensitivity of theses responses to MET support the presence of 5-HT_1-like receptors in addition to 5-HT_2 receptors mediating vasoconstriction in EDV. The low potency of 8-OH-DPAT suggests the 5-HT_1-like receptor in EDV does not resemble the 5-HT_{1a} receptor.

JHW was in receipt of a Wellcome Vacation Scholarship. This work was funded by the Home of Rest for Horses.

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172P PHARMACOLOGICAL CHARACTERISATION OF THE RELAXATION RESPONSE TO 5-HT IN THE RAT TERMINAL ILEUM USING THE 5-HT_4 RECEPTOR ANTAGONIST GR 113808

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5-Hydroxytryptamine (5-HT) and related indoles produce a relaxation response in the rat terminal ileum which is mediated via a tropisetron sensitive site (Tuladhar *et al.*, 1991). In the present study we have further characterised the 5-HT receptor mediating the relaxant response using the highly selective and specific 5-HT_4 receptor antagonist GR 113808 (Grossman *et al.*, 1993).

Isolated ileal segments (2-3 cm in length, 3-20 cm from the ileo-ceacal junction) were obtained from female Hooded Lister rats (200-300g) and mounted in 10 ml organ baths containing oxygenated (95%O_2 / 5%CO_2) Krebs-Henseleit solution (37°C) containing methysergide (10 μM) and atropine (0.1 μM). The tissues were allowed to equilibrate for 60-90 min with washout every 15 min. Changes in tissue tension were recorded isometrically. Concentration-response curves to 5-HT and other agonists were obtained in a cumulative manner using a 2 min contact time. A comparison of the effects with and without antagonist was carried out using paired preparations. GR 113808 was added to the reservoir except when its effect on the tissue tone was measured. p-Chlorophenylalanine was given intraperitonially to rats in a volume of 0.5 ml with 5% Tween 80 as a vehicle.

In the presence of methysergide and atropine, GR 113808 (10-100 nM) caused parallel dextral shifts of the concentration-response curve to 5-HT. The slope of the Schild regression was not significantly different from 1 (1.31, 95% confidence limit 0.9-1.72). A pK_B value of 8.95 (95% confidence limit 8.81-9.10) was estimated by constraining the slope to unity. GR 113808 (10 nM) caused a dextral shift of the 5-methoxytryptamine, 5-carboxamidotryptamine and renzapride concentration-response curves in a parallel manner from which apparent pK_B values of 8.83±0.11 (n=6), 8.82±0.22 (n=4), and 8.97±0.21 (n=4) respectively were calculated, considering a slope of unity. GR 113808 increased the tone of the quiescent ileum by 154±63 mg (n=6, 10 nM GR 113808), 146±45 (n=6, 30 nM GR 113808) and 150±33 mg (n=6, 100 nM GR 113808). This phenomena was also observed using tropisetron (217±29 mg, n=6 with 10 μM tropisetron) and SDZ 205-557 (103±33 mg, n=6 with 3 μM SDZ 205-557). The increase in tone due to GR 113808 was abolished after pretreating the rats with intraperitoneal p-chlorophenylalanine (n=3, 300 mg/kg for 6 days).

The competitive antagonism of the 5-HT response by GR 113808 with an estimated pK_B value of 8.95 indicates that the relaxation response is mediated by the 5-HT_4 receptor (Grossman *et al.*, 1993). Agonist independence of the pK_B value of GR 113808 further substantiates the 5-HT_4 receptor involvement. The abolition of the GR 113808 induced increase in tone after pretreatment of the rats with p-chlorophenylalanine indicates the presence of an endogenous 5-HT_4 tone in the rat terminal ileum. However, its presence does not adversly effect the measurment of relaxation responses to the exogenous addition of 5-HT agonists and the antagonism by GR 113808.

We thank the ORS Awards Scheme for providing support to B. R. Tuladhar and Sandoz and Glaxo for the gifts of drugs.

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173P INFLUENCE OF ENDOGENOUS 5-HT FUNCTION ON THE 5-HT$_4$ RECEPTOR MEDIATED RELAXATION IN THE RAT TERMINAL ILEUM

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The 5-HT$_4$ receptor in the rat ileum mediates a relaxation response to the administration of exogenous 5-hydroxytryptamine (5-HT) (Tuladhar *et al.*, 1991). In the present study we investigate the influence of an increase or decrease in endogenous 5-HT function on the response to exogenous 5-HT.

Isolated ileal segments (2-3 cm in length, 3-20 cm from the ileo-caecal junction) were obtained from female Hooded Lister rats (200-300g) and mounted in 10 ml organ baths containing oxygenated (95%O_2 / 5%CO_2) Krebs-Henseleit solution (37°C) containing methysergide (1-10 μM) and atropine (0.1 μM). The tissues were allowed to equilibrate for 60-90 min with washout every 15 min for the first 45 min. Changes in tissue tension (from an initial tension of 0.6-0.75g) were recorded isometrically. Concentration-response curves to 5-HT and other agonists were obtained in a cumulative manner using a 2 min contact time. A comparison of the effects of agonists and antagonists was carried out using paired preparations. GR 113808 and fluoxetine were equilibrated for at least 30 min before the concentration-response curves to exogenous 5-HT were obtained. p-Chlorophenylalanine (p-CPA) was given intraperitonially (300 mg/kg for 3 days) to rats in a volume of 0.5 ml with 5% Tween 80 as a vehicle. Triplicate samples of ileal mucosa were taken for the determination of 5-HT and 5-hydroxyindole acetic acid (5-HIAA) by HPLC-ECD.

The selective 5-HT reuptake inhibitor fluoxetine (10 μM) abolished the relaxation response to exogenous 5-HT, a concentration of 1 μM was effective in only 2 out of 5 tissues examined and the use of a lower concentration of fluoxetine (0.1 μM) was ineffective. The treatment with fluoxetine failed to abolish the relaxation induced by noradrenaline (1 μM) (n=5). The treatment with p-CPA (n=4) reduced 5-HT and 5-HIAA tissue levels by 88% and 77% respectively in ileal mucosa with a statistically non-significant trend to increase the maximum relaxation response to 5-HT (n=4). The selective 5-HT$_4$ receptor antagonist GR 113808 (30 nM) (Grossman *et al.*,1993) when administered alone increased the basal tone of the tissue and caused a dextral shift in the concentration-response curve to 5-HT (apparent pK_B value of 8.95±0.13, n=4). The abolition by fluoxetine of the response to 5-HT was prevented in the tissues taken from animals pretreated with p-CPA (n=4), the response being potentiated by a factor of 4 (pEC_{50} with and without fluoxetine was 7.52±0.16 and 6.91±0.84 respectively, n=4).

Fluoxetine, although more potent in central tissues, is a selective blocker of neuronal uptake of 5-HT in enteric neurons (Gershon & Jonakait, 1979) and is known to enhance synaptic concentrations of 5-HT. It is hypothesised that the ability of fluoxetine to abolish the relaxant effects of exogenous 5-HT in the rat ileum reflects an endogenous 5-HT induced desensitization of the 5-HT$_4$ receptor, a receptor known to rapidly desensitise in response to agonist challenge (Ansanay *et al.*, 1992). The ability of the p-CPA treatment to prevent the action of fluoxetine supports this hypothesis. Similar conclusions may be drawn from the ability of GR 113808 to increase basal tone. The presence of an endogenous 5-HT$_4$ tone may contribute to lower estimates of pK_B values in the rat ileum as compared to 5-HT$_4$ binding assays (Grossman *et al.*,1993).

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174P DIFFERENTIAL ACTIVITY OF (R) AND (S)-ZACOPRIDE TO MODIFY 5-HT$_4$ RECEPTOR MEDIATED RELAXATION OF THE RAT TERMINAL ILEUM

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5-Hydroxytryptamine (5-HT) produces a relaxation response in the rat terminal ileum which is mediated via a 5-HT$_4$ receptor (Tuladhar *et al.*, 1991). In the present study we have used this preparation to investigate the agonist and antagonist activities of (R) and (S)-zacopride at the 5-HT$_4$ receptor using SDZ 205-557 as a reference 5-HT$_4$ receptor antagonist (Buchheit *et al.*, 1992).

Isolated ileal segments (2-3 cm in length, 3-20 cm from the ileo-caecal junction) were obtained from female Hooded Lister rats (200-300g) and mounted in 10 ml organ baths containing oxygenated (95%O_2 / 5%CO_2) Krebs-Henseleit solution (37°C) containing methysergide (1 μM) and atropine (0.1 μM). The tissues were allowed to equilibrate for 60-90 min with washout every 15 min for the first 45 min. Changes in tissue tension (from an initial tension of 0.6-0.75g) were recorded isometrically. Concentration-response curves to 5-HT and other agonists were obtained in a cumulative manner using a 2 min contact time. A comparison of the effects of agonists and antagonists was carried out using paired preparations. Antagonists were equilibrated for at least 30 min. Schild plots using the isomers of zacopride were obtained by the method of Van Rossum, 1963.

In the presence of methysergide and atropine, SDZ 205-557 (0.3-3 μM) caused parallel dextral shifts of the concentration-response curve to 5-HT. The slope of the Schild regression was not significantly different from 1 (0.96, 95% confidence limits 0.54-1.37). A pK_B value of 7.00 (95% confidence limits 6.90-7.09) was estimated by constraining the slope to unity.

Both (R) and (S)-zacopride acted as partial agonists with a maximum response 56.6±3.6% (n=5) and 48.6±9.1% (n=5) respectively of that of 5-HT. pEC_{50} values for (R) and (S)-zacopride (5.32±0.05 and 5.76±0.15 respectively, n=5 both) were statistically different ($p<0.05$ Student's t test unpaired two tailed) with the (S)- isomer being 2.75 times more potent. In the presence of (S)-zacopride (1-100 μM) concentration-response curves to 5-HT were dextrally shifted with reductions in the maximum response. The Schild regression with dose ratio calculated from the half maximal responses to 5-HT gave a slope not significantly different from 1 (1.03, 95% confidence limits 0.83-1.23). Imposing the unity constraint a pK_B value of 6.17 (95% confidence limits 5.68-6.66) was obtained. However, such concentration-dependent antagonism could not be demostrated with (R)-zacopride (1-100 μM), the Schild slope with a dose ratio calculated from the half maximal responses to 5-HT being significantly ($p<0.05$) less than 1(0.64, 95% confidence limits 0.38-0.89, pA_2 5.37).

The competitive antagonism of the 5-HT response by SDZ 205-557 with the estimated pK_B value of 7.00 and inactivity of ondansetron (Tuladhar *et al.*, 1991) indicate that the relaxation response is mediated by the 5-HT$_4$ receptor (Buchheit *et al.*, 1992). Both isomers of zacopride acted as partial agonists in this system with (S) isomer being slightly more potent. The (S) isomer was also a competitive antagonist of the effects of the 5-HT but this could not be demostrated for (R)-zacopride. The agonist/antagonist actions of the isomers may contribute to their effects 'in vivo'(Barnes *et al.*, 1992).

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175P AN ONDANSETRON-RESISTANT COMPONENT OF 5-HT-EVOKED DEPOLARIZATION IS PRESENT IN THE VAGUS NERVES OF THE RAT AND MOUSE BUT NOT OF THE GUINEA-PIG OR RABBIT

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We have previously described a component of 5-hydroxytryptamine (5-HT)-evoked depolarization of the rat and mouse isolated vagus nerves that is resistant to blockade by the 5-HT_3 receptor antagonist ondansetron and that has characteristics of a 5-HT_4 receptor mechanism (Rhodes *et al.*, 1992; Coleman & Rhodes, 1992; Coleman & Rhodes, 1993). We have extended this work to include the guinea-pig and rabbit, species widely used in studies of 5-HT_3 receptor antagonists.
Extracellular recording of depolarization of the cervical vagus nerve was carried out using a "grease-gap" technique (Rhodes et al., 1992). Male, Sprague Dawley rats (180-220 g) were killed either with a blow to the head and cervical dislocation or by cervical dislocation following anaesthesia with pentobarbital (70 mg kg^{-1} i.p.) Male mice (T.O. strain, 20-50 g) and male guinea-pigs (Dunkin-Hartley, 300-330 g) were killed by cervical dislocation. Male rabbits (New Zealand Whites, 1.9-2.2 kg) were killed with pentobarbital (60 mg kg^{-1}i.v.). The vagus nerves were placed in two chamber tissue baths, one chamber being perfused (3 ml min^{-1}) with Krebs' solution (2.5 mM $CaCl_2$, at 27°C) to which drugs were added. Contact time for 5-HT was 3 min with a 10-15 min washout between doses. Two non cumulative concentration-response curves (CRC's) were obtained in each tissue, the second CRC being obtained after 40 min equilibration with ondansetron at 100 nM (rat and mouse), 30 nM (rabbit) or 3 μM (guinea-pig).

Table 1.

Species	Control 5-HT CRC's (without ondansetron)		First phase of the 5-HT CRC (with ondansetron)			
	Max. depolarization (μV)	Geometric mean EC_{50} (μM)	First phase max. (% initial max)	Geometric mean EC_{50} (μM)	Apparent pA_2 of ondansetron	n
Rat	402 (364-440)	0.50 (0.40-0.63)	15.5 (12.6-19.2)	0.10 (0.05-0.20)	8.6 (8.5-8.8)	8
Rat*	335 (247-423)	0.67 (0.28-1.59)	13.1 (2.8-23.5)	0.12 (0.07-0.19)	8.8 (8.3-9.3)	3
Mouse	270 (240-300)	0.39 (0.21-0.57)	17.6 (14.3-20.9)	0.04 (0.014-0.10)	8.3 (8.0-8.9)	16
Rabbit*	1095 (919-1271)	3.72 (2.75-5.13)	No first phase		9.5 (8.8-10.2)	4
Guinea-pig	644 (370-919)	4.79 (3.32-7.19)	No first phase		6.8 (6.7-7.0)	9

* Anaesthetised with pentobarbital (95% confidence limits)

In the absence of ondansetron, CRC's to 5-HT in all species appeared monophasic with characteristics summarized in Table 1. In the presence of ondansetron, CRC's from mice and from rats killed either during or without pentobarbital anaesthesia were biphasic with an initial (first phase) component resistant to ondansetron. The maxima and the EC_{50} values of the first phase were similar in each of these groups (Table 1). No evidence of this component was detected in preparations from the guinea-pig or rabbit, CRC's remaining monophasic in the presence of ondansetron. The apparent pA_2 values for ondansetron calculated from that part of the curve displaced to the right by ondansetron suggest a difference in 5-HT_3 receptors in the guinea-pig (Lattimer et al., 1989).

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176P 5-HT_3 AND 5-HT_4 RECEPTORS MEDIATE THE SECRETORY EFFECTS OF 5-HT IN RAT DISTAL COLON

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5-Hydroxytryptamine$_4$ (5-HT_4) receptors have previously been implicated in chloride secretion in rat distal colon (Bunce *et al.*, 1991). The lack of a selective antagonist for this class of receptors, however, precluded the conclusive characterisation of this response. Recently, GR113808 has been identified as a potent and selective antagonist at the 5-HT_4 receptor (Grossman *et al.*, 1993) and this compound has been used to further characterise the 5-HT receptor mediating chloride secretion in rat colon

Sections of distal colon were obtained from Wistar rats (150-200g) and mucosa, stripped of its muscle layers, was set up in Ussing chambers and bathed with oxygenated Krebs solution at 37°C. Tissues were voltage clamped and changes in the short-circuit current (scc) measured. Cumulative agonist concentration-response curves (CRC) were constructed. Antagonists were preincubated for 30min prior to the test agonist CRC.

In rat distal colon 5-HT (0.3-100μM) stimulated reproducible increases in scc (for successive curves, concentration ratio CR=1.4 (1.2-1.8); n=6). The 5-HT response was partially inhibited by both TTX (0.3μM) and ondansetron (1μM) with depressions in maximum to 33.7±8.9% (n=4) and 46.0±2.4% (n=6) respectively. In the presence of TTX, responses to 5-HT were unaffected by granisetron (1μM; CR=1.6 (1.1-2.5) n=8). 5-Methoxytryptamine (5-Meot; 1-100μM) increased scc to 72.8±6.1% of the 5-HT standard with an equipotent molar ratio of 2.4 (1.2-4.6)(n=6). To investigate the effects of 5-HT receptor antagonists the relatively selective 5-HT_4 receptor agonist, 5-methoxytryptamine, was used. Tropisetron (3μM) produced a rightward shift of the 5-Meot CRC (CR=14.5 (13.8-15.2) at the EC_{30} level; n=3) to give a pK_B of 6.6±0.1. The maximum response was depressed to 63.0±10.1%. 5-Meot responses were also inhibited by GR113808 (1nM) (CR at the EC_{40} level=8.0 (1.7-37.5)) with a pK_B of 9.8±0.2 (n=4). The maximum response was depressed to 72.5±8.7%. GR113808 (10 and 100nM) completely blocked responses to 5-Meot up to 100μM (n=4-7). The effects of both tropisetron (3μM) and GR113808 (1μM) were selective for 5-Meot as they had no effect on response to forskolin (CRs=1.7 (1.1-2.6) and 1.2 (0.8-1.9)(n=4) respectively).

The present results suggest that 5-HT produces its secretory effects partly through the 5-HT_3 receptor, since responses could be inhibited by ondansetron, and partly through the 5-HT_4 receptor as shown by the apparent pK_B values determined for both tropisetron and GR113808.

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177P INVESTIGATION OF THE RECEPTOR MECHANISM MEDIATING THE RELAXATION RESPONSE TO 2-METHYL-5-HYDROXYTRYPTAMINE IN GUINEA-PIG ASCENDING COLON

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In the course of investigating the relaxation responses to 5-hydroxytryptamine (5-HT) in guinea-pig ascending colon (GPAC) (Elswood & Bunce, 1992), we determined the effect of the 5-HT_3 receptor agonist, 2-methyl-5-hydroxytryptamine (2-Me5-HT). The purpose of this study was to characterise the receptor mechanism involved in this latter response.

The muscle layers of GPAC were dissected from the attached mucosa and set up in the longitudinal plane under a resting tension of 0.5-0.7g. Tissues were bathed in Krebs-Henseleit solution gassed with 95% O_2/5%CO_2 at 37°C. Single doses of 2Me-5HT (or other agonist) were added, and washed out when a maximum response had been achieved, with 10min between applications. Experiments were carried out in paired tissues; one control and one equilibrated with antagonist for 30min.

In preliminary experiments 2-Me5-HT stimulated mainly contraction responses although, in some tissues, small relaxations occurred at 1μM. In the presence of ondansetron (2μM) or alosetron (30μM) clear relaxation responses occurred, and these were further studied in tissues precontracted with carbachol (0.3μM). Under these conditions 2-Me5-HT produced reproducible concentration-related relaxations with EC_{50} values of 50 (41-61) and 46 (41-52)μM in the two tissues (n=6), with a maximum inhibition of the carbachol tone of 100% at 100μM. Phentolamine (0.1 and 0.3μM) produced rightward shifts of the 2-Me5-HT concentration-response curve with respective concentration ratios of 2.6 (1.8-3.7) and 7.9 (5.2-11.8), from which apparent pK_B values of 7.2±0.1 (n=6) and 7.5±0.1 (n=8) were calculated. Prazosin (6 and 30nM) also produced rightward shifts of these responses with respective concentration-ratios of 2.8 (1.8-4.4) and 5.2 (3.1-8.9), from which apparent pK_B values of 8.4±0.2 (n=8) and 8.1±0.1 (n=10) were calculated.

These data indicate that the relaxation responses to 2-Me5-HT were mediated directly or indirectly by α_1-adrenoceptors. This conclusion is supported by the observation that in GPAC precontracted with carbachol (0.3μM), the α_1-adrenoceptor agonist, methoxamine, induced relaxations with an EC_{50} value of 3.8 (3.4-4.3)μM. Prazosin (6, 30, 100nM) produced parallel shifts of these concentration-response curves with a pA_2 value of 8.8±0.1 [slope = 0.8 (0.7-1.0)].

An interaction of 2-Me5-HT with α-adrenoceptors has previously been reported *in vivo* (Dalton et al., 1986). In this latter study 2-Me5-HT induced vasopressor responses in DOCA hypertensive rats pretreated with a 5-HT_3 receptor antagonist and, as in the present study, these response were blocked by phentolamine.

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178P EVALUATION OF THE MOUSE ISOLATED SUPERFUSED DISTAL COLON AS A FUNCTIONAL 5-HT_3 RECEPTOR CONTAINING PREPARATION

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Binding studies have revealed the presence of 5-HT_3 recognition sites in the gastrointestinal tract of a number of species (Pinkus *et al.*,1989). The present study evaluates the mouse isolated, superfused colon as a novel, functional model for the assessment of 5-HT_3 receptor agonists and antagonists.

Segments of distal colon (2cm) from male CRH mice were set up in superfusion chambers (Coleman & Nials, 1989) under 1g initial tension. Tissues were superfused with Krebs containing ketanserin, mepyramine, phentolamine (all 1μM) and indomethacin (2.8μM), gassed with 95% O_2, 5%CO_2 at 32°C. Following 1hr equilibration, during which resting tension was reset to 1g, tissue viability was assessed with KCl (10μl; 20μmoles); tissues responding with contractions of <200mg were rejected. Agonist dose-response curves were obtained by injecting 10μl bolus doses of 2-Me-5-HT, 1-phenylbiguanide (PBG), or 1-(m-chlorophenyl)-biguanide, (mCPBG) into the superfusion stream at 5min intervals, until a maximum contractile response was obtained. In each experiment, two agonist dose-response curves were constructed 3h apart. Antagonists or antagonist vehicle were added to the superfusion reservoir and equilibrated with the tissue for 3h before re-challenging with agonist. Contractile responses were expressed as % response to KCl. Agonist potency was expressed as the ED_{50}, (dose (moles) of each compound required to produce 50% of its maximum contractile effect). Values stated are arithmetic mean ± s.e.mean.

2-Me-5-HT, PBG (both 0.3-10nmoles), and mCPBG (0.03-3nmoles) caused transient, dose-related contractions of the mouse colon. PBG and 2-Me-5-HT were equiactive; (ED_{50} 0.7(0.5-1.2) and 0.7(0.5-0.9)nmoles; maximum contraction 87±13 and 88±9% KCl, n=9 and n=16 respectively) and mCPBG approximately 10 times more potent; (ED_{50} 0.06(0.02-0.13) nmoles) with a lower maximum response (61±11% KCl, n=10). Two consecutive dose-response curves to 2-Me-5-HT 3h apart were superimposable. Contractions to 2-Me-5-HT were unaffected by ketanserin, methysergide or atropine (all 1μM) but abolished in the presence of tetrodotoxin (1μM). The selective 5-HT_3 receptor antagonists ondansetron (3-30nM) and granisetron (1-10nM) attenuated contractions elicited by 2-Me-5-HT. Ondansetron caused a small shift to the right in the agonist dose-response curve with a marked suppression of the maximum response, dose-ratio (DR), measured at ED_{50} as described above 1.7±1.1; 35±6%KCl at 30nM, (n=13) whilst granisetron caused a larger shift with less of a reduction in the maximum response, DR 27.4±14.8; 66±8%KCl at 3nM, (n=14).

Thus, 2-Me-5-HT causes neurogenic, non-cholinergic contractions of the mouse distal colon. Contractions elicited by the 5-HT_3 receptor agonists, PBG, mCPBG and 2-Me-5-HT and antagonism of the latter by the 5-HT_3 receptor antagonists ondansetron and granisetron, but not by 5-HT_1 or 5-HT_2 receptor blockade demonstrates the existence of a functional 5-HT_3 receptor-mediated response in this tissue. This preparation may prove useful in characterising functional attributes of 5-HT_3 receptor ligands. However, the insurmountable nature of the 5-HT_3 receptor antagonism in this tissue may preclude its use in quantitative pharmacological studies.

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179P EVALUATION OF A COMPUTER ASSISTED LEARNING PROGRAM ON INTESTINAL MOTILITY

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The computer assisted learning (CAL) program on "Intestinal Motility", previously demonstrated to the BPS (Dewhurst *et al.*, 1992) has now been evaluated by comparison with a tutored demonstration (DEM) using students for whom the program was originally designed, ie. second year preclinical students at CXWMS. The 1992/93 practical class "circus" cohort of 156 students were randomised to either DEM or CAL groups (n = 14-16) for their gut practical. In each of 10 sessions a common introduction was given, in which the purpose and procedures of the evaluation were explained. Students were requested not to confer with members of the other group before handing in their work. They were assured that, although their answers would be scored for the purpose of the evaluation, the scores would not contribute to their practical course mark; this would be determined separately. The DEM and CAL groups each had a tutor present throughout the 90min class. Tutors were randomised to take DEM and CAL groups on different occasions so that individual characteristics should not influence the outcome of the evaluation.

The present report consists of a comparison of resources utilised for the two teaching methods and of the students' learning as assessed by their scores for answers to five of the 20 questions they were given. These were selected to test the students' extant knowledge linking peristalsis, acetylcholine, neostigmine and atropine, their understanding of the preparation, their data interpretation ability and the extent of their progress through a common sequence of tests.

Resources: The DEM required 56h of technician's and 24h of tutor's time, whereas the CAL requirements were 4h and 15h respectively. Apparatus (DEM) and computers (CAL) were available from stock. Rats for the DEM cost £50 whereas the CAL program cost £120, but this could be used again. Other costs were similar for both groups.

Learning: Answers from 52 DEM and 53 CAL students were amenable to analysis. The scores (percentages of students giving satisfactory answers) for the five questions respectively were 95%, 70%, 86%, 63% and 34% (mean 69%) for the DEM group and 98%, 50%, 72%, 51% and 19% (mean 58%) for the CAL group.

Thus, both DEM and CAL methods produced satisfactory results, with CAL being much less expensive but also a little less effective than DEM. Subsequent discussion suggested that guided private study of the CAL program followed by tutorial support could be more effective than the method used here.

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180P ANTI-INFLAMMATORY ACTIVITY IN EXTRACTS FROM *GUAIACUM OFFICINALE* WOOD CUTS

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Extracts of the heartwood of *G. officinale* or *G. sanctum*, family Zygophyllaceae, are indicated for the treatment of rheumatoid arthritis (British Herbal Pharmacopoeia, 1983). We have tested extracts of *G. officinale* for anti-inflammatory activity. Suspensions of the extracts in 10 % acacia mucilage were administered, p.o. 18 h and 2 h prior to the induction of carrageenan-induced rat paw oedema (Winter *et al.*, 1962). Oedema was monitored at 1 h intervals as percent increase in paw thickness over 6 h. The maximal and total oedema responses (measured as area under the time-course curves - A.U.C) over 6 h were determined.

Table 1: Effect of 70 % ethanolic extract of guaiacum on carrageenan-induced rat paw oedema:

	Dose	Response as % mean control value	
Treatment	(mg kg-1)	Max. swelling	A.U.C.
G. officinale			
	100.0	95.4 ± 12.2	90.1 ± 7.3
	200.0	84.5 ± 4.7*	76.5 ± 7.2*
	400.0	69.0 ± 4.1*	60.6 ± 5.1*
Indomethacin	2.5	54.0 ± 5.7*	41.6 ± 4.5*

Results are mean ± s.e.m., n = 6. Asterisks indicate differences from vehicle controls at $p < 0.05$, (one way ANOVA plus Newman-Keuls range test)

The extract obtained by boiling under reflux in 70 % ethanol produced a dose-related inhibition of carrageenan-induced oedema (Table 1). Soxhlet extraction with petrol ether (60 - 80) provided a fraction which, administered at 400 mg kg-1 p.o., inhibited ($p < 0.05$) both carrageenan-induced oedema and neutrophil infiltration into the carrageenan-induced lesions (monitored as myeloperoxidase activity; Bradley *et al*, 1982) by 19.6 % and 26.6 %, respectively. Further Soxhlet extraction with ethyl acetate or methanol produced fractions inactive against carrageenan-induced oedema when given at 400 mg kg-1 p.o. Daily administration of the aqueous ethanol extract (200 mg kg-1, p.o.) to adjuvant arthritic rats (Pearson, 1956) significantly ($p < 0.05$) reduced the mean maximal tibio-tarsal joint swelling produced during 18 days in the non-injected limbs to 71.1 ± 6.5 % of the mean inflamed control value. Guaiacum extracts thus contain lipophilic anti-inflammatory substances which are potentially anti-arthritic.

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MD was supported by the Association of Commonwealth Universities

181P ANTI-INFLAMMATORY EFFECTS OF PF 10040 IN RABBIT AND GUINEA-PIG SKIN

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The experimental compound PF 10040 has been previously shown to inhibit PAF-induced oedema formation in rabbit skin (Rossi *et al*, 1992) and PAF-induced hyperresponsiveness and eosinophil accumulation in neonatally immunized rabbits (Herd *et al*, 1993). In this study, we have investigated the effects of locally-injected PF 10040 on oedema formation and ^{111}In-neutrophil accumulation induced by different mediators of inflammation in rabbit skin and on ^{111}In-eosinophil accumulation and oedema formation in a passive cutaneous anaphylaxis (PCA) reaction in guinea pig skin.

Rabbit blood neutrophils were purified on a discontinuous percoll gradient, labelled with $^{111}InCl_3$ and injected i.v. ($2x10^7$cells/animal) together with ^{125}I-human serum albumin (HSA). In some experiments, the animals received only ^{125}I-HSA. Five minutes later, inflammatory stimuli were given i.d. with or without PF 10040. The drugs were mixed prior to i.d. injections. After thirty minutes, blood samples were obtained, the rabbits killed and skin sites punched-out. Similarly, guinea pig peritoneal eosinophils were purified on percoll gradient and labelled with $^{111}InCl_3$. ^{111}In-eosinophils ($5x10^6$ cells/animal) were injected i.v. followed by i.d. injection of antigen in sites previously sensitized 16-20 h earlier with an IgG_1-rich sera. Two hours later, animals were sacrificed and skin sites punched out.

In rabbit skin, PF 10040 (10^{-7}moles/site) significantly inhibited oedema formation induced by bradykinin (n=6), leukotriene B_4 (LTB_4, n=9), zymosan-activated plasma (n=3), FMLP (n=10) and PAF (n=25). For example, oedema formation induced by LTB_4 (5×10^{-10}mol/site) was 44.9 ± 6.5 µl and 31.1 ± 5.0 µl in the absence and presence of PF 10040, respectively ($P<0.01$). Similarly, oedema responses induced by PAF (10^{-9} moles/site) were reduced by 51% (Saline, 9.8 ± 1.1; PAF, 44.7 ± 4.9 µl; PAF + PF 10040, 26.8 ± 3.6 µl; $P< 0.01$). ^{111}In-neutrophil accumulation induced by PAF, but not by FMLP or LTB_4, was significantly inhibited by PF 10040 (PAF, 1926 ± 660; PAF + PF 10040, 791 ±335, $P < 0.05$, n=4). In the guinea pig skin, PF 10040 (10^{-7}moles/site) inhibited oedema formation and ^{111}In-eosinophil accumulation in a PCA reaction by 48% ($P < 0.01$) and 25% ($P < 0.05$, n=6), respectively, whereas the PAF antagonist WEB 2086 (10^{-7}moles/site) had no effect.

These results suggest that locally administered PF 10040 possesses anti-inflammatory activity in rabbit and guinea pig skin but this activity may not be related exclusively to PAF antagonism. The ability of the drug to inhibit oedema formation and the accumulation of eosinophils may be relevant to conditions where these inflammatory changes have a pathophysiological role. Further studies are however necessary to clarify the mechanism of action of this compound.

This work was supported by the National Asthma Campaign, the Wellcome Trust and Purdue Frederick Co., USA.
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182P CHARACTERISATION OF RECEPTORS MEDIATING BRADYKININ AND des-Arg^9-BK-INDUCED PAW OEDEMA IN THE MOUSE

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It has been reported previously that bradykinin (BK) will cause oedema in the mouse (Mantione and Rodriguez, 1990). Two receptors, B_1 and B_2, have been classified which potentially could mediate this response (Regoli and Barabé, 1980). The objective of the present study was to characterise the receptor(s) involved in oedema induced by BK and des-Arg^9-BK in the mouse.

Glaxo-bred male CR/H strain mice (25-50g) were injected intraplantar (i.pl.) into the left hind paw with 25µl of either BK or des-Arg^9-BK (0.1-100 nmole). Paw oedema was measured 15 min later using a modified plethysmometer (Ugo Basile). Groups of 6-8 animals were treated with single doses of agonist and 5 groups used to construct a response curve. Subsequently in the same animals BK or des-Arg^9-BK was injected into the right hind paw, and oedema measured. Antagonists were either administered subcutaneously (s.c.) or coinjected into the paw with agonists. Comparisons were made between response curves obtained in left and right paws. Injection of BK or des-Arg^9-BK caused significant and dose-related oedema ED_{50} 1.5 (0.1-25) nmole (n=12) and 4.3 (1.7-11.5) nmole (n=12) respectively. Time-course studies demonstrated that the oedema peaked at 15 min and had regressed by 120 min. Response curves to single agonists obtained in left and right paws were not significantly different.

The B_2-receptor antagonist, HOE 140 (Wirth et al., 1991) (500 nmole kg^{-1} s.c.) caused a 11.7 fold rightward shift of the BK dose-response curve, but had no significant effect on the response to des-Arg^9-BK. Conversely, the putative B_1-receptor antagonist, des-Arg^{10}-HOE 140 (Rhaleb et al., 1992) (500 nmole kg^{-1} s.c.) caused a 4.3 fold rightward shift of the des-Arg^9-BK response curve, without significantly affecting BK-induced oedema. The B_1-receptor antagonist des-Arg^9-Leu^8-BK caused significant dose related oedema ED_{50} 20.1 (8.8-64.6) nmole (n=12) being approximately 10-fold weaker than BK. The effect of both agents was completely inhibited by coadministration of the histamine H_1-receptor antagonist mepyramine (50 nmole i.pl.). Aspirin (200 mg kg^{-1}; i.p. 30 min pretreatment) inhibited both BK and des-Arg^9-BK-induced oedema, equivalent to 100 fold and 55.5 fold rightward shifts respectively. These data suggest that BK and des-Arg^9-BK-induced oedema in the mouse are mediated by B_2 and B_1 receptors respectively and that prostaglandins are likely to play a significant role in both responses. Additionally the ability of des-Arg^9-Leu^8-BK to induce oedema demonstrates that its use in establishing a role for B_1 receptors *in vivo* should be undertaken with caution.

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183P RELATIVE POTENCY OF NONSTEROID ANTI-INFLAMMATORY DRUGS AS INHIBITORS OF CYCLOOXYGENASE-1 OR CYCLOOXYGENASE-2

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Cyclooxygenase (COX) exists in at least two different isoforms. COX-1 is constitutive and present in cells under physiological conditions, whereas COX-2 is induced by cytokines or endotoxin *in vitro* (Maier, et al., 1990;Akarasereenont, et al., 1993) and under inflammatory conditions *in vivo* (Mitchell et al., 1993a). Nonsteroid anti-inflammatory drugs (NSAIDs), such as aspirin inhibit COX activity (Vane, 1971), a property which most likely accounts for their shared therapeutic effects by inhibition of COX-2 and side effects by inhibition of COX-1. Aspirin and indomethacin are selective inhibitors of COX-1 (Mitchell et al., 1993b). Here we investigate the effects of various NSAIDs as inhibitors of COX-1 or COX-2 in intact cells. Bovine aortic endothelial cells (COX-1) or endotoxin-activated (1 μg/ml for 12 h) J774.2 macrophages (COX-2) were cultured in 96 well plates and COX activity measured by the conversion of arachidonic acid (30 μM) to 6-keto prostaglandin $F_{1\alpha}$ by cells which were preincubated with NSAIDs for 30 min as described previously (Mitchell et al., 1993b).
Table 1 shows the IC50 values (μg/ml) of NSAIDs on COX-1 or COX-2 activity in intact cells. The means ± the s.e. of the mean is given, the number of determinations (wells) is shown in parenthesis. * mean data from Mitchell et al., 1993b.

Drug	COX-1 (IC50)	COX-2 (IC50)	ratio COX-2/COX-1
Piroxicam	0.0005± 0.002	0.3 ± 0.7	600 (n=12)
Tolmetin	0.04 ± 0.003	7 ± 1.22	175 (n=9)
*Aspirin	0.3 ± 0.2	50 ± 10	166 (n=12)
Sulindac	0.4 ± 0.28	40 ± 23.33	100 (n=9)
*Indomethacin	0.01 ± 0.001	0.6 ± 0.08	60 (n=12)
Tolfenamic acid	0.0003± 0.0007	0.005± 0.0019	16.67 (n=12)
BW755c	0.65 ± 0.26	1.2 ± 0.78	1.85 (n=9)
Carprofen	3 ±0.41	3 ±1.72	1 (n=9)
Diclofenac	0.5 ± 0.21	0.35 ± 0.15	0.7 (n=9)
Naproxen	2.2 ± 0.98	1.3 ± 2.2	0.59 (n=12)
BF389	0.15 ± 0.01	0.03 ± 0.01	0.2 (n=12)
Ticlopidine	0.15 ± 0.05	inactive	(n=9)

For activity against COX-2, piroxicam displayed the lowest selectivity ratio and BF389 (an experimental NSAID; Bendele et al., 1992) the highest. The use of COX-1 and COX-2 activity in intact cells may provide useful information for the development of selective inhibitors of COX-2 with improved potency as anti-inflammatory drugs and fewer side effects.

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184P ROLE OF PAF IN LEUCOCYTE ACCUMULATION IN ALLERGIC AND NON-ALLERGIC INFLAMMATORY REACTIONS IN GUINEA-PIG SKIN

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PAF is a potent inflammatory mediator which can stimulate neutrophils and eosinophils in vitro inducing chemotaxis, adhesion and degranulation (Braquet *et al.*, 1987). The aim of the present study was to investigate the role of PAF, using the potent PAF antagonist UK-74,505 (Cooper *et al.*, 1990), in the accumulation of neutrophils (casein-elicited) and eosinophils (horse serum-elicited) and plasma leakage in guinea-pig skin. Cell accumulation and oedema formation were simultaneously measured by the local accumulation of i.v. injected ^{111}In-leucocytes and ^{125}I-albumin, respectively (Faccioli *et al.*, 1991). The inflammatory reactions investigated were elicited by the exogenous administration of PAF, LTB_4 and ZAP (as a source of C5a des Arg) or by endogenously released mediators in response to zymosan and in a passive cutaneous anaphylaxis (PCA) reaction. The PCA reaction was induced as previously described using an anti-BGG antisera as the sensitizing antibody (Weg *et al.*, 1990). Intradermal PAF (10^{-9}mol/site) and ZAP (100%) induced the accumulation of ^{111}In-neutrophils, ^{111}In-eosinophils and oedema formation over a 2h in vivo test period. LTB_4 ($5x10^{-10}$mol/site) induced ^{111}In-eosinophil accumulation but was weak in causing neutrophil accumulation and oedema formation. A pronounced accumulation of ^{111}In-leucocytes and plasma leakage was also observed at PCA sites. UK-74,505, (0.5 and 2.5mg/kg i.v.), virtually abolished leucocyte accumulation and oedema formation induced by PAF but had no significant effect on the responses induced by LTB_4, ZAP and the PCA. However, UK-74,505 (0.5mg/kg) significantly inhibited the plasma leakage induced by zymosan without affecting cell accumulation. At the higher dose of the antagonist (2.5mg/kg),^{111}In-eosinophil accumulation and plasma leakage were significantly suppressed by UK-74,505 (Table1).

Table 1 Effect of UK-74,505 (2.5 mg/kg) on ^{111}In-eosinophil accumulation (mean ± s.e. mean, n=6 pairs of animals). Difference from control responses (2-way ANOVA) is denoted: *$p<0.05$, **$p<0.01$

	SALINE	PAF 10^{-9}mol/site	LTB_4 $5x10^{-10}$mol/site	PCA	zymosan 100μg/site
CONTROL	0.58±0.13 x10^3	3.62±0.70 x10^3	9.10±4.33 x10^3	5.13±1.37 x10^3	2.04±0.48 x10^3
UK-74,505	0.46±0.07 x10^3	0.53±0.075 x10^3 **	5.64±2.11 x10^3	6.55±1.28 x10^3	1.08±0.23 x10^3 *

The results indicate that, whilst PAF is not involved in the inflammatory responses induced by LTB_4, ZAP and a PCA reaction, it does appear to have a role in the responses elicited by zymosan.

Supported by: The Wellcome Trust, National Asthma Campaign and Conselleria de Cultura, Educacion y Ciencia (Generalitat Valenciana).
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185P THE RELATIONSHIP BETWEEN ARTERIAL OXYGEN TENSION AND PROSTACYCLIN LEVELS *IN VIVO*

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The vascular endothelium has been proposed to act as an oxygen sensor, modulating its output of vasoactive substances in response to changes in oxygen tension (Pohl, 1990). In this study we have investigated the relationship between oxygen tension and prostacyclin levels in arterial blood.

Arterial blood was obtained from patients in intensive care. Patients were sedated, but were receiving no other drugs, and had been maintained on a constant supply of oxygen for at least 30min prior to sampling. Two blood samples were collected in heparinised tubes via a radial arterial line; one sample was used for immediate blood gas analysis and the other frozen at -20°C. Frozen samples were thawed, ethanol precipitated and the supernatant applied to activated Sepak C18 columns in 0.1M phophate buffer, pH 7.2. Columns were washed with 5ml each of water and hexane and the sample eluted in 5ml 1% methanol in ethylacetate. Following evaporation to dryness, the sample was resuspended in phophate buffered saline and 6-keto-$PGF_{1\alpha}$, the stable hydrolysis product of prostacyclin, assayed by radioimmunoassay as previously described (Hunt et al., 1992). Recovery of 6-keto-$PGF_{1\alpha}$ from the solvent extraction procedure was 50%.

The partial pressures of oxygen in the 36 samples obtained ranged from 8.83-58.3kPa. There was a marked and significant positive correlation between oxygen tension and the measured concentration of 6-keto-$PGF_{1\alpha}$ ($r=0.936$), which ranged from 0.23 nmol/ml blood at the lowest oxygen concentration to 2.77 nmol/ml blood at the highest oxygen concentration. There was no correlation between 6-keto-$PGF_{1\alpha}$ concentration and any of the other parameters obtained, such as CO_2 tension, pH, platelet count, blood pressure, heart rate, patient age or weight.

Thus there seems to be a marked positive correlation between arterial oxygen concentration and prostacyclin production. The significance of this relationship and the source of prostacyclin remains to be determined.

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186P REGULATION OF EXPRESSION OF INTERCELLULAR ADHESION MOLECULE-1 ON THE CULTURED HUMAN UMBILICAL VEIN ENDOTHELIAL CELL LINE EA-hy-926

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Increase in expression of endothelial cell (EC) adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) contributes to the accumulation of leukocytes at sites of inflammation (Springer, 1990; Williams and Hellewell, 1992). Although it is known that cytokines such as tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) increase expression of ICAM-1 and other EC adhesion molecules (Pober *et al.*, 1986), the intracellular regulatory mechanisms that control this expression remain largely unknown. In the present study we examined the role of mRNA transcription, protein synthesis and protein kinase C (PKC) activation on TNF-α + IFN-γ-induced expression of ICAM-1 on the human umbilical vein EC line EA-hy-926 (Edgell *et al.*, 1983). In addition, we sought to investigate the effect of dexamethasone on the expression of this adhesion molecule, since although glucocorticoids are potent and widely used anti-inflammatory agents the mechanisms by which they reduce inflammation are unclear.

ICAM-1 was detected by an enzyme-linked immunosorbant assay using a mouse anti-human ICAM-1 primary antibody and a peroxidase-linked goat anti-mouse secondary antibody. Antibody binding was quantified using a peroxidase-sensitive substrate, azinobis (3-ethyl-benz-thiazoline) sulfonic acid. Results were expressed as fold increase (± S.E.M) in absorbance (measured at 405 nm) for cytokine-treated cells over untreated controls. Statistical analysis was performed using one-way ANOVA followed by the Tukey-Kramer Multiple Comparison Test. Results were deemed significant if $P < 0.05$.

Basal expression of ICAM-1 was not detected on EA-hy-926. Stimulation of EA-hy-926 with a combination of TNF-α (10 ng/ml)and IFN-γ (1000 units/ml) resulted in a time-dependent increase in the expression of ICAM-1, reaching maximal levels by 24 h (3.67 ± 0.13-fold increase; n=8). This level of expression was maintained (in the presence of TNF-α + IFN-γ) at 48 h (3.87 ± 0.18-fold increase; n=6). However, under these conditions neither E-selectin or vascular cell adhesion molecule-1 were detected. The TNF-α + IFN-γ-induced increase in ICAM-1 expression detected at 6h was abolished when cells were stimulated in the presence of either actinomycin D (5 μg/ml) or cycloheximide (10 μg/ml). Furthermore, ICAM-1 expression was also abolished on co-incubation with the PKC inhibitor H7 (50 μM). However, treatment of these cells with TNF-α +IFN-γ in the presence of dexamethasone at concentrations of either 1 μM (3.94 ± 0.14 fold increase; n=12) or 10 μM (4.24 ± 0.11 fold increase; n=8) did not significantly alter TNF-α + IFN-γ-stimulated ICAM-1 expression at 24h (3.85 ± 0.10 fold; n=12; $P > 0.05$).

In conclusion, these results demonstrate that ICAM-1 expression in EA-hy-926 stimulated with TNF-α + IFN-γ is dependent on mRNA transcription, protein synthesis and protein kinase activation C, but cannot be down-regulated by dexamethasone.

This work was funded by the Clinical Research Committee of the Royal Brompton National Heart and Lung Hospital. PGH is supported by the National Asthma Campaign.

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187P A SIMPLE, RELIABLE METHOD FOR THE MEASUREMENT OF ALBUMIN CLEARANCE ACROSS CULTURED ENDOTHELIAL CELL MONOLAYERS

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Measurement of albumin clearance across endothelial cell monolayers is being increasingly used as an *in vitro* model of plasma protein extravasation. We have now developed a simple reliable method based on a technique described by Cooper *et al.* (1987).

Bovine pulmonary artery endothelium (CPAE, obtained from the ECACC) was cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 17% foetal calf serum (FCS). Cells were seeded onto polycarbonate filter assemblies (Costar Transwell, 12 assemblies in a 12-well plate, $1cm^2$ filter, 0.4μm pore size, pre-soaked in DMEM/FCS for at least 1hr) at 3-6 times confluent density, and cultured for 7 days. The assemblies were rinsed with phosphate-buffered saline, and then 0.4ml and 1.5ml bathing medium (DMEM containing 25mM HEPES, 1.2mM $NaHCO_3$, $40mg.ml^{-1}$ bovine serum albumin, 0.001% silicone anti-foaming agent, but without FCS or phenol red, pH adjusted to 7.4, 37°C, pre-gassed with air) was added to the inner *luminal* and outer *abluminal* chambers respectively. Evans blue (50μl, $10mg.ml^{-1}$ in bathing medium) was added to the luminal chambers, and under these conditions >99.9% of Evans blue was bound to albumin. 50μl samples were removed from the luminal chambers, and diluted (1:10) before measurement of absorbance (600nm). The assemblies were placed in a water bath (37°C, exposed to air) and left for 20 min (*equilibration* period). The assemblies were gently swirled and 100μl samples removed from the abluminal chambers; this was replaced with an equivalent volume fresh bathing medium. The assemblies were left for 1hr (*basal* period) before samples were again removed from the abluminal chambers (as previously). Drug (or vehicle) was added to the luminal chambers. The assemblies were left for 1hr (*treatment* period) before samples were once more removed from the abluminal chambers. Absorbances (600nm) of the samples was measured in a 96-well plate, corrected for dilution and replacement of medium, and the albumin clearance rate (theoretical volume of the luminal chamber cleared of Evans blue-labelled albumin per unit time) calculated for the basal and treatment periods (as described below).

Clearance = (Abluminal volume x Increase in abluminal absorbance) / (Luminal absorbance x Time)

Clearance rate across cell-free filters was $72.3\mu l.hr^{-1}$ (s.d.=$9.8\mu l.hr^{-1}$, n=12). Basal clearance rate across cell-coated filters was $6.2\mu l.hr^{-1}$ (s.d.=$3.6\mu l.hr^{-1}$, n=48). In vehicle-treated controls there was no significant increase in clearance between basal and treatment periods ($p>0.05$, Student's paired *t*-test, n=28), demonstrating the stability of the permeability barrier. Thrombin ($0.01\text{-}100U.ml^{-1}$) increased clearance in a concentration-dependent manner, with the highest concentration of thrombin increasing clearance by $8.0\mu l.hr^{-1}$ (s.e.mean $0.8\mu l.hr^{-1}$, $p<0.001$ vs. vehicle, unpaired t-test, n=8).

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188P SULPROSTONE IS A POTENT CONTRACTILE AGENT ON HUMAN PULMONARY ARTERY *IN VITRO*: POSSIBLE THERAPEUTIC IMPLICATIONS

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Sulprostone, a metabolically-stable prostaglandin E analogue (see Collins & Djuric, 1993), contracts the human myometrium and is used to arrest atonic post-partum haemorrhage. Following two severe cardiovascular incidents associated with intravenous infusions of sulprostone at the Prince of Wales Hospital, Shatin, it was decided to investigate the actions of sulprostone on human blood vessels, commencing with isolated pulmonary artery preparations.

Pulmonary lobar arteries (3 - 5 mm in diameter) were dissected from apparently healthy lung tissue obtained from patients undergoing lobectomy for lung cancer. Isometric tension changes of the circular muscle in vessel rings, 3 - 4 mm wide, were recorded (Krebs, 95% O_2/5% CO_2, 37°C, indomethacin 1 μM). Sulprostone was a highly potent contractile agent in all 30 preparations obtained from 9 patients. However considerable variation in sulprostone concentration-response curves was observed, due at least in part to tachyphylaxis. Threshold concentrations ranged from 0.35 to 6.0 nM, EC_{50} values from 1.8 to 10 nM, and maximum responses from 25 to 65% of the U-46619 maximum. Responses to the TP-receptor agonist U-46619 (EC_{50} = 3 - 10 nM) remained constant over 6h. Sulprostone contractions were not blocked by the TP-receptor antagonist EP 169 (200 nM) (Tymkewycz *et al.*, 1991); the pA_2 against U-46619 was approximately 8.3.

A preliminary examination of other PGE analogues showed that PGE_2, misoprostol and GR 63799X all contracted the pulmonary artery and were about 5 - 15, 5 - 10 and 15 times less active than sulprostone respectively. Above 100 nM PGE_2 activates TP-receptors. The specific FP-receptor agonist fluprostenol did not contract the preparation (up to 2 μM), excluding the involvement of FP-receptors. This is important, since we have have observed, contrary to popular belief, that sulprostone appears to be a moderately potent FP-receptor agonist. On the dog and cat iris sphincter preparations sulprostone is only 9.1 (EC_{50} = 18.2 nM, n = 6) and 6.7 (EC_{50} = 58 nM, n = 2) times less active than $PGF_{2\alpha}$ respectively.

Our agonist potency rankings would suggest that sulprostone and the other PGE analogues studied contract the human pulmonary artery via activation of EP_3-receptors (see TIPS Receptor Nomenclature Supplement, 1993). A similar but less sensitive contractile system has been described for the rabbit renal artery (Ahluwalia *et al.*, 1988). The findings reported here emphasize the need to consider pulmonary vasoconstriction when EP_3 agonists are used as therapeutic agents.

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189P VASCULAR PHARMACOLOGY AS AN INDEX OF LIVER TRANSPLANT SURVIVAL

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Clinical parameters of storage injury show University of Wisconsin buffer (Belzer & Southard, 1988) to be beneficial in liver transplantation and morphological studies relate the coincidence of graft failure from storage injury with endothelial cell death. Endothelial preservation might be evaluated pharmacologically by ACh induced arterial smooth muscle relaxation.Rabbit aortic rings were stored for 0(tested on harvesting)), 2, and 8 days in UW, KB, or saline at 4°C prior to mounting in an isolated organ bath and preconstriction with noradrenaline. Relaxation to ACh after storage for 2 or 8 days was greater in UW than KB, whereas there was little or no response after saline storage. UW therefore appears to preserve greater endothelial integrity. In contrast, direct smooth muscle relaxation to SNP after 8 days storage was less in UW than KB. We postulated that this reduction in SNP relaxation in UW stored rings might also reflect greater endothelial integrity, with increased basal NO competition for cGMP giving diminished SNP responses. However,this reduction in SNP induced relaxation in UW stored rings was unaltered by L-NAME and SNP responses on freshly harvested rings, when endothelial integrity is maximal, were also unaltered by L-NAME. Therefore competition between SNP and NO for cGMP does not explain this paradox. Pharmacological analysis of endothelial preservation might reflect those clinical parameters of graft failure due to storage injury. However, as UW preservation is believed to be non-selective, it is unclear why smooth muscle relaxation is reduced in UW rings.

% dilatations (mean ± S.E.) to ACH and SNP following storage in UW or KB after preconstriction with 2 x 10-6M noradrenaline.

Day	Drug	Log/Mol	UW	KB	UW vs KB
2	ACh	-6.5	74.86 ± 6.62	51.74 ± 5.44	P = 0.031
2	ACh	-6.0	87.52 ± 6.81	65.23 ± 6.50	P = 0.047
8	ACh	-7.0	51.25 ± 5.77	28.36 ± 7.12	P = 0.02
8	ACh	-6.5	65.61 ± 4.63	40.69 ± 8.73	P = 0.015
8	ACh	-6.0	82.45 ± 4.99	43.06 ± 9.88	P = 0.002
8	SNP	-7.5	26.04 ± 2.38	44.50 ± 9.93	P = 0.030
8	SNP	-7.3	33.60 ± 2.18	73.95 ±1.55	P = 0.0001
8	SNP	-7.0	57.62 ± 4.00	96.87 ±3.13	P = 0.002
8SNP+L-NAME		-7.0	40.66 ± 4.70	65.11 ± 8.78	P = 0.035
8SNP+L-NAME		-6.4	76.23 ± 1.86	90.99 ± 3.34	P = 0.005
8SNP+L-NAME		-6.0	98.80 ± 1.12	104.08± 2.04	P = 0.046

UW = University of Wisconsin solution, KB = Krebs-Büllbring buffer, .ACh = acetylcholine, SNP = sodium nitroprusside.NO = nitric oxide. L-Name = Nw - Nitro-L-arginine methyl ester. UW vs KB = Student's, 2 tailed, unpaired t-test.

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190P THE MECHANISM OF INHIBITION OF GUINEA-PIG EOSINOPHIL CYCLIC NUCLEOTIDE PHOSPHODIESTERASE (PDE) BY PDE IV SELECTIVE INHIBITORS

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Selective inhibitors of PDE IV (high affinity, cyclic AMP specific PDE) may be potentially useful therapeutic agents in the treatment of asthma as these compounds exhibit both bronchodilator and anti-inflammatory activities (Torphy and Undem 1991). We recently reported that the PDE IV selective inhibitors, rolipram and denbufylline, showed differences in the mechanism of inhibition of PDE IV isoenzymes prepared from brain and tracheal smooth muscle (Shahid *et al.*, 1993). In view of the importance of the anti-inflammatory activity of PDE IV inhibitors, in the present study we have examined the mechanism of inhibition for rolipram, denbufylline and Org 20241 on guinea pig eosinophil PDE IV. Guinea pig peritoneal eosinophils were harvested from male animals sensitised to human serum and processed on a discontinuous percoll gradient to obtain 90-95% cell purity. The final cell pellets from 2-3 harvests (~3 x 10^8 cells) were pooled and homogenised in buffer containing (mM): Bistris 20, dithiothreitol 1, benzamidine 2, phenylmethanesulphonyl flouride 0.1, EDTA 2, NaCl 50, (pH 6.5) and centrifuged at 100,000g for 1 hr. The pellet fraction was resupended in 3 ml of homogenisation buffer containing 250 mM sucrose and was used as the source of PDE IV. PDE activity was assayed (Shahid *et al.*, 1991) and kinetic parameters determined (Shahid *et al.*, 1993) as described before. The cyclic AMP PDE activity in guinea-pig eosinophils was predominantly membrane bound (96.6±2.4%, n = 4) and insensitive to the regulators Ca/calmodulin and cyclic GMP (1 μM). Most of the particulate PDE activity was characterised as being due to PDE IV, however, Org 9935 (3 μM) which is a potent PDE III selective inhibitor (Shahid *et al.*, 1991) produced 10-15% inhibition indicating the minor presence of PDE III. Thus all subsequent kinetics experiments were performed in the presence of 3 μM Org 9935. Eosinophil PDE IV showed Michaelis-Menten kinetics with respect to cyclic AMP (Km = 5.6 μM and Vmax = 7.78 pmol/min/10^6 cells) with a Hill coefficient of 1.18. Rolipram (Ki = 1.66; Ki' = 1.67 μM), denbufylline (Ki = 1.47; Ki' = 1.32 μM) and Org 20241 (Ki = 3.94; Ki' = 5.29 μM) produced concentration dependent increases in Km and decreases in Vmax indicating a mechanism of mixed inhibition of PDE IV. In contrast 3-isobutyl-1-methylxanthine (Ki = 22.8 μM) produced increases in Km only and thus acted as a competitive inhibitor.

In conclusion, the mechanism of inhibition of eosinophil PDE IV by selective inhibitors is similar to that found in tracheal smooth muscle. These data suggest that there may be a greater homology between PDE IVs from these tissues than brain PDE IV which is competitively inhibited by these inhibitors (Shahid *et al.*, 1993).

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191P EFFECT OF THIAZIDE-LIKE DRUGS ON CALCIUM HOMEOSTASIS IN CULTURED GUINEA PIG VASCULAR SMOOTH MUSCLE CELLS

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Hydrochlorothiazide (HTZ) and cicletanine (CIC) possess weak Ca^{2+} antagonistic properties in guinea pig mesenteric arteries while indapamide (IND) is a more potent Ca^{2+} antagonist (Calder *et al*, 1993). This study examines the effects of thiazides on peptide-induced Ca^{2+} mobilisation in cultured guinea pig vascular smooth muscle cells (VSMC).

Guinea pig mesenteric arteries were carefully dissected free, cleaned and cut into small pieces (explants) and placed into tissue culture flasks. This tissue was cultured using minor modifications of the explant technique of Chamley Campbell *et al* (1979) in Dulbecco's medium containing 25mM HEPES buffer supplemented with 10% fetal calf serum (FCS), penicillin (100μg/ml) and streptomycin (100μg/ml). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. VSMC migrated out from the explants to form a monolayer which was subcultured by trypsinisation. The standard 10% FCS medium was replaced with 0.4% FCS medium to growth arrest the cells 72 hours before use. VSMC were loaded with fura2-AM for 90min, trypsinised, centrifuged and resuspended in physiological saline solution containing zero calcium (PSS^0). Fluorescence was measured using a PTI Deltascan dual wavelength spectrofluorimeter at excitation wavelengths 340/380nm, with emission at 510nm. The cell suspension (2.5ml) was placed in a quartz cuvette in the Deltascan chamber. The effects of the peptides endothelin (ET: 100nM) and vasopressin (VP: 100nM) on $[Ca^{2+}]_i$ in the presence and absence of a 10μM concentration of HTZ, IND or CIC were studied. At the end of each experiment the maximum and minimum 340/380 ratios were determined by addition of Triton X (0.1%) and EGTA (5mM). From these values the concentration of peptide-induced increase in $[Ca^{2+}]_i$ mobilised by the peptides could be calculated (Grynkiewicz *et al*, 1985).

The effects of HTZ, CIC and IND on peptide-induced Ca^{2+} mobilisation is illustrated in table 1.

Table 1. Effects of drugs on ET and VP-induced Ca^{2+} mobilisation

	Control	HTZ	CIC	IND
ET	158 (100-277)	98 (73-131)	112 (64-178)	66* (20-115)
VP	74 (60-132)	40* (39-53)	58 (39-103)	47* (31-64)

Values are in nM and represent rise of intracellular Ca^{2+} concentration in absence (control) and presence of thiazides due to stimulation by ET and VP, results are medians of four separate experiments, numbers in parentheses represent the range in nM. * $P<0.01$, concentration significantly different from control concentration (Kruskal-Wallis and Wilcoxon range tests).

CIC had no significant effect on either ET or VP-induced Ca^{2+} mobilisation in guinea pig VSMC. HTZ significantly reduced VP but not ET-induced rise in intracellular Ca^{2+} and IND reduced both ET and VP-induced Ca^{2+} signals. This implies that HTZ and IND interfere with $[Ca^{2+}]_i$ mobilisation but the mechanisms involved have yet to be clarified.

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192P VASOCONSTRICTOR-STIMULATED TYROSINE PHOSPHORYLATION OF MITOGEN-ACTIVATED PROTEIN KINASE IN VASCULAR SMOOTH MUSCLE CELLS IN CULTURE

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The intra-cellular signalling mechanisms by which growth factors and vasoconstrictors stimulate vascular smooth muscle cell (VSMC) proliferation have not yet been fully identified. Increasing evidence suggests that tyrosine phosphorylation of a number of cystolic enzymes plays an important role in transducing initial second messenger signals to the cell division apparatus (Cadena & Gill, 1992). In particular, mitogen-activated protein kinase (MAP kinase) may play an important role as an integration switch in a phosphorylation cascade (Nishida & Gotoh, 1993). We therefore examined the ability of classical G-protein linked vasoconstrictors, α-Thrombin (α-Thr), Angiotensin II (AII) and Endothelin-1 (ET-1), and platelet derived growth factor (PDGF) to stimulate tyrosine phosphorylation of cellular proteins in particular, the pp42kDa and pp44kDa isoforms of MAP kinase.

VSMCs were cultured up to 10 passages and quiesced before experiments by serum deprivation for 48 hours. Agonist induced tyrosine phosphorylation was examined over a time course of one hour. Autoradiographs of anti-phosphotyrosine western blots were quantified using scanning densitometry. MAP kinase pp42kDa and pp44kDa were identified after western blotting using an anti-MAP kinase monoclonal antibody (All antibodies were supplied by Affiniti Research Products Ltd, Nottingham, England).

AII (100nM) stimulated the tyrosine phosphorylation of both pp42kDa and pp44kDa isoforms of MAP kinase. Phosphorylation peaked at 5 min, decreasing to 22%±8 of the maximum by ten min before returning to basal levels (11.8% ± 7) at 15 min. An EC_{50} value of 0.22nM ± 0.1nM (n=3) was obtained for AII-stimulated tyrosine phosphorylation. α-Thr (1.35 units) stimulation resulted in a similar pattern of tyrosine phosphorylation, maximal at 5 min decreasing to basal levels at 15 min (6%±2.7). In contrast ET-1 (100nM) stimulated a bi-phasic response with a maximum signal at 5 min, decreasing to basal levels at 15 min (13.9%±6) then increasing again to 30%±11.4 at 30 and 60 min. Stimulation with PDGF (30ng/ml) resulted in a sustained tyrosine phosphorylation of MAP kinase. The response peaked around 5-10 min with phosphorylation slowly decreasing to 46.7%±7 by 60 min. An EC_{50} value of 1.86ng/ml ± 0.5ng/ml (n=3) was obtained for PDGF stimulated tyrosine phosphorylation. 100nM 12-O-tetradecanoyl phorbol 13-acetate (TPA) also stimulated the sustained tyrosine phosphorylation of pp42kDa and pp44kDa isoforms of MAP kinase. This response was abolished by the protein kinase C inhibitor, Ro-318220 (Davis *et al* 1989), with an IC_{50} value of 1.74μM ± 0.42μM (n=4). Stimulation with AII in the presence of Ro-318220 (10μM) resulted in an apparent delayed, but greater and prolonged phosphorylation/activation of MAP kinase (2min; 84.4%±25.8, +Ro-318220 43.4%±25: 5min; 100%±15.6, +Ro-318220 121.7%±7: 15min; 15.3%±10, +Ro-318220 43.3%±15.6). Similiar results were obtained with α-Thr, ET-1 and PDGF. However, 24 hours pretreatment with TPA (100nM) to down regulate protein kinase C effectively abolished all responses to G-protein linked vasoconstrictors.

These results imply that the tyrosine phosphorylation of MAP kinase can be regulated by vasoconstictors which utilise the PKC pathway as well as by growth factors, such as PDGF, which activate receptors with intrinsic tyrosine kinase activity, the two pathways converging upstream of MAP kinase. It is possible that the activation of both pathways is required for the regulation of cell growth and division.

We wish to thank; Dr A Crilley (Dept Medical Biochemistry, Royal Infirmary Glasgow) for the gift VSMCs and Dr G Lawton (Roche Products Ltd, Welwyn Garden City, Herts) for the gift of Ro-318220. This work was funded by the Medical Research Council and the National Asthma Campaign

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193P EFFECTS OF ENDOTHELIAL INTEGRITY AND SURGICAL PREPARATION ON RESPONSES OF HUMAN SAPHENOUS VEIN AND INTERNAL THORACIC ARTERY TO VASOPRESSIN

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Human saphenous vein (HSV) grafts are commonly used in coronary artery bypass surgery (CABS). Damage to the vein grafts including de-endothelialization has been attributed to the surgical preparation involved (Angelini et al. 1989). The internal thoracic artery (ITA) is a preferred conduit to HSV as it requires little surgical preparation and shows better long term patency. In order to assess the reactivity of the two vessels, we have examined the responses in vitro of HSV which had been freshly isolated (FHSV) or subjected to surgical preparation (PHSV) and ITA to vasopressin a vasoconstrictor present at high concentrations during CABS (Knight et al. 1986). HSV which had previously functioned as a graft was obtained from five patients undergoing reoperation and its contractile responses to vasopressin determined.
FHSV, PHSV or ITA (2-4mm rings) were mounted in organ baths in Krebs-Henseleit solution under initial tension of 30 or 60 mN respectively. Changes in tension were recorded isometrically. Rings were tested for their response to potassium chloride (KCl, 90mM) and Arg-vasopressin (AVP) (10^{-10}-10^{-7} M). Integrity of endothelium was assessed by relaxation caused by bradykinin (10^{-5}M), of rings precontracted by phenylephrine (10^{-5}M).

Maximal responses of vascular rings to AVP as % of KCl response (shown as medians with interquartile range)

	ITA+end	ITA-end	FHSV+end	FHSV-end	PHSV+end	PHSV-end	Replaced grafts
E_{max}	36(11,77)	46(25,74)	15(9,23)*	34(20,50)	18(10,25)*	35(18,42)	26(4,32)
n	18	17	32	10	15	18	5

* P<0.01, +end vs -end, Mann Whitney test.

The presence of endothelium had no influence on responses of ITA to AVP. However, responses of HSV to AVP were augmented in the absence of endothelium. Since surgical preparation of HSV often leads to damaged endothelium (18 out of 33 PHSVs did not relax to bradykinin) our results would suggest that surgical preparation prior to grafting, may potentiate responses of HSV to AVP in vivo. Furthermore, the responses of the replaced grafts indicate that vascular responsiveness is maintained throughout the life of the graft.

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194P EFFECT OF PLATELET-DERIVED GROWTH FACTOR ON TONE AND INTRACELLULAR Ca^{2+} IN ISOLATED RABBIT EAR ARTERY

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Platelet derived growth factor (PDGF) is a potent mitogen for vascular smooth muscle cells (Ross 1989). PDGF also contracts vascular smooth muscle (Berk & Alexander 1989), although the mechanism of this effect is unclear. This study examines the role of Ca^{2+} in the contractile action of PDGF in isolated rabbit ear artery.

Branches of rabbit ear artery were mounted in a myograph containing physiological saline at 37°C and bubbled with 95% O_2, 5% CO_2 for simultaneous measurement of force and intracellular Ca^{2+} (Cai) (Jensen et al., 1992). Arteries were loaded with the Ca^{2+}-sensitive fluorescent dye, fura-2, by incubation with fura-2AM (5μM) for 3h at 37°C. Tissues were illuminated at 340nm and 380nm using a spectrofluorimeter connected to an inverted microscope, and emission at 510nm was measured. Force and fluorescence data were acquired on-line at 4-10Hz using an A/D converter linked to an IBM PC. Data are presented as means ±s.e.means of 3-5 experiments.

Table: The effect of various stimulants on force and Cai in rabbit ear artery.

Stimulant	Δforce (mN)	ΔCai (340/380 ratio units)
K^+ (118mM)	8.8±3.9	1.78±0.39
NA (10μM)	9.8±4.9	1.70±0.37
PDGF (100pM)	1.1±0.2	0.75±0.27
NA + PDGF	2.2±0.2	0.70±0.31

Δforce and ΔCai were calculated by subtracting resting force or Cai from the peak response to the stimulant. Resting 340/380 ratio was 0.4±0.06.

PDGF (100pM) caused a small contraction associated with a rise in Cai which was usually sustained. Following a stable contraction with noradrenaline (NA; 10μM) PDGF produced similar rises in Cai but force generation tended to be increased (table). PDGF- induced contraction and rises in Cai were abolished by removal of extracellular Ca^{2+} (n=3) or by 5μM (-)202 791, a dihydropyridine calcium antagonist (n=3).

PDGF increases Cai and contracts rabbit ear artery. These effects depend on influx of extracellular Ca^{2+}, probably through a voltage-operated calcium channel. The mechanism by which PDGF causes opening of calcium channels remains to be established.

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195P DOSE-RELATED RESPONSES TO VASOACTIVE AGENTS IN THE HEPATIC ARTERY AND PORTAL VEIN OF THE ISOLATED DUAL-PERFUSED RABBIT LIVER: EFFECTS OF PROLONGED PERFUSION

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The vascular reactivity of the isolated dual-perfused rabbit liver preparation remains stable for 2.5 hours of perfusion (Alexander et al 1992). Recent experiments studying the effect of hypoxia upon vascular reactivity required perfusion periods beyond 4 hours (Alexander et al 1993). The apparent similarity in the effect of hypoxia on different vasodilators, occurring late in these experiments, prompted a re-evaluation of the vascular responses of the model during extended periods of perfusion in order to exclude the effects of preparation fatigue. This was evaluated by testing the reproducibility of vasodilator responses in the hepatic arterial (HA) and portal venous (PV) beds to HA bolus dose injections of acetylcholine (10^{-8} moles), adenosine (10^{-7} moles), ATP (10^{-7} moles), and sodium nitroprusside (10^{-7} moles) at 30 minute intervals during 5 hours of perfusion.

Circuit details and additional methodology have been provided elsewhere (Alexander et al 1992) but in summary, 5 livers (109 ± 8.1g) from 2.5-3.5 kg New Zealand White rabbits were perfused with Krebs-Bullbring buffer, through the HA and PV at 25 and 75 ml min^{-1} 100g $liver^{-1}$ respectively and oxygenated with a 95% O_2/ 5% CO_2 gas mixture. The tone of the preparation was raised with 5 x 10^{-7} to 10^{-6} M noradrenaline, generating perfusion pressures of 147 ± 11 and 4.9 ± 0.7 mmHg in the HA and PV respectively, which were maintained throughout the 5 hour perfusion period (a further 2 experiments demonstrated that the unconstricted pressures did not increase over the 5 hours). The pO_2 during the perfusion remained constant at 491 ± 7 mmHg and after passage through the liver decreased to 191 ± 7 mmHg. The pH and pCO_2 of the perfusate also remained constant at 7.33 ± 0.01 and 31.4 ± 0.5 mmHg respectively.

Liver viability was asessed by the maintenance of hepatic oxygen uptake, bile volume production and release of potassium and lactate into the liver effluent. Hepatic oxygen uptake was constant during the perfusion at 0.89 ± 0.03 ml O_2 min^{-1} 100g $liver^{-1}$. Bile volume production decreased significantly from 7.0 ± 1.8 to 3.3 ± 0.9 ml h^{-1}* consistent with the interruption of the enterohepatic recirculation of bile salts. There was no change in the release of potassium or lactate from the liver. There was no statistically significant change in the HA responses to any of the vasodilators, but there was a decrease in the amplitude of the response to acetylcholine after 2.5 hours and adenosine after 4 hours. There was a significant increase in the PV constrictor response to acetylcholine after 3 hours (1.7 ± 0.4, 2.2 ± 0.3* and 2.9 ± 0.5* mmHg at 1, 3 and 4 hours respectively) and to ATP after 2 hours (2.1 ± 1.0, 2.8 ± 0.8*, 4.2 ± 1.3* mmHg at 1, 2 and 5 hours respectively).

It is concluded that the isolated dual-perfused rabbit liver preparation remains viable for 5 hours of perfusion with Krebs-Bullbring buffer and that the HA resistance sites remain sufficiently oxygenated under these conditions to preserve their function. However, responses of PV resistance sites alter during prolonged perfusion in a similar manner to that seen during hypoxia. These changes may represent 'fatigue' of the preparation or may be indicative of resistance site hypoxia (especially post-sinusoidal) occurring during 'oxygenated' perfusion. (* $P < 0.05$ compared to 1 hour, Student's paired t-test. All results shown are mean ± se.)

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196P CONTRACTILE, CHRONOTROPIC AND ELECTROPHYSIOLOGICAL EFFECTS OF THE NOVEL VASODILATOR, FLOSEQUINAN

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Although the potent vasodilating effect of flosequinan is well characterized, the positive inotropic action is more variable between studies and less well understood. In *in vitro* studies, while flosequinan appears to have a positive inotropic effect which is dose-dependent, this effect has not always been observed in the therapeutic concentration range, in the region of 10^{-5}M (Yates, 1991; Perreault *et al.*, 1992). This inotropic action does not appear to be mediated by a cyclic AMP-dependent mechanism (Perreault *et al.*, 1992). However, at higher concentrations of the drug, cyclic AMP mediated actions do seem to be involved as a result of weak inhibition of phosphodiesterase isoenzymes. The present study examines the contractile, chronotropic and electrophysiological effects of flosequinan in cardiomyocytes from adult Sprague-Dawley rats (200-250g). Contractile function, as measured by mechanical cell shortening (dL) and expressed as a percentage of diastolic length, was monitored using video microscopy (n=5). The effects of flosequinan on action potential (AP) characteristics were studied in left ventricular papillary muscles using conventional intracellular microelectrode techniques, and the frequency of beating was investigated in isolated spontaneously beating atria of the same species (n=10). Comparisons were tested using analysis of variance followed by a multiple range (Dunnet's) or paired Students 't' test.

Flosequinan produced a significant positive contractile effect ($p<0.05$), only at the maximum concentration used (10^{-3}M), which was 24% greater than the mean basal dL value of 7.5±0.17(S.E.)%. However, the effect of flosequinan to increase the frequency of beating was concentration-dependent and significant over the range 10^{-5}M to $3x10^{-4}$M; flosequinan, at a concentration of $3x10^{-4}$M, maximally increased the mean frequency of beating by 31% of the predrug value (255±15 beats min^{-1}). Enoximone, at a concentration of 10^{-5}M, produced a maximal effect on frequency of beating, 30% greater than the predrug value, whereas the increase by flosequinan, at an equimolar concentration, was 23%. These values are in contrast to the lack of increase produced by digoxin (10^{-9}M to $3x10^{-8}$M). Flosequinan did not have any effect on resting membrane potential, amplitude, or maximum upstroke velocity in isolated left ventricular papillary muscle. However, at the maximum concentration used (10^{-3}M), flosequinan decreased the duration of the AP at 10%, 50% and 75% repolarization ($p<0.05$). In the absence of flosequinan, the durations of the AP at 10%, 50% and 75% repolarization were 3.9±0.5, 13.2±0.8 and 23.3±1.7 ms, respectively; in the presence of the drug, the corresponding parameters were 2.2±0.3, 8.8±1.0 and 18.0±2.7 ms, respectively. The metabolite of flosequinan, BTS 53554, did not produce any changes in action potential characteristics over the concentration range, 10^{-5}M-10^{-3}M.

In conclusion, flosequinan (10^{-5}M to $3x10^{-4}$M) had a more pronounced effect on chronotropy than on contractile function, which was influenced significantly only at elevated concentrations (10^{-3}M). The shortening of the duration of the AP at this elevated concentration, is compatible with a cyclic AMP-mediated action noted with other selective PDE III inhibitors.

This work was supported by the British Heart Foundation

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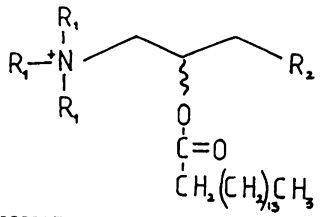
197P STRUCTURE-ACTIVITY RELATIONSHIPS OF SOME VASODILATOR DERIVATIVES OF PALMITOYL CARNITINE IN THE ISOLATED RAT HEART CORONARY CIRCULATION

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Esterification of palmitoyl carnitine (PC) produces compounds which are potent and long lasting coronary vasodilators (Criddle et. al. 1990). This study investigates the importance of the ester grouping, as well as the size of the alkyl groups attached to the quaternary nitrogen on the degree of vasodilator activity, by comparing potencies of various structural derivatives of PC in the isolated perfused rat heart.
Hearts from male Wistar rats (300-350g) were perfused via the aorta at 10ml./min. using 3.2mM K^+ Krebs-Henseleit gassed with 95% O_2, 5% CO_2. The low K^+ concentration raises the coronary tone and gives a greater scope for a vasodilator response. Bolus dose-response curves were constructed to each compound, allowing the preparation to recover to baseline perfusion pressure between drug additions.

Table 1 shows the various structural modifications and relative activities of the PC derivatives tested, indicated by the ED_{50}, and the degree of vasodilation achieved at the optimum dose (measured as %relaxation from basal perfusion pressure, %RELAX.).

	R1	R2	ED_{50} nmol.	%RELAX. (dose)
A	3x CH_3	$COOCH(CH_3)_2$	0.44±0.09**	56.3±6.3 (3nmol.)
B	3x CH_3	CH_3	1.06±0.07	35.7±1.7 (10nmol.)
C	3x CH_2CH_3	CH_3	0.26±0.09**	35.0±8.0 (1nmol.)
D	3x $CH_2CH_2CH_3$	CH_3	0.60±0.13*	35.8±3.7 (10nmol.)
E	2x CH_3* *(ie.uncharged)	CH_3	INACTIVE	--



All data shown as mean±sem, n=4-5.
*p<0.05 **p<0.01 compared to compound B, 1 way ANOVAR.

These results show that compounds B,C & D, without an ester group at the R2 position are active vasodilators, although the degree of vasodilation achieved (indicated by %RELAX.) is less than that of A, the isopropyl ester. The uncharged tertiary amine derivative, compound E, was inactive as a vasodilator. Three ethyl groups at R1 (C) conferred greater vasodilator potency, as measured by ED_{50} than three methyl or propyl groups (B & D). It is also worth noting that all the derivatives of PC shown above, with the exception of the isopropyl ester, showed a transient vasoconstriction preceding the vasodilator response.
Thus, it appears that the absence of a negatively charged carboxylic acid group, rather than the presence of the ester grouping confers vasodilator activity on these palmitoyl carnitine derivatives. Furthermore, the size of the alkyl groups attached to the quaternary nitrogen profoundly affects vasodilator activity, and a quaternary nitrogen ie. a positive charge is essential for vasodilator activity.

Supported by Sigma-Tau, Rome.

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198P CAROTID ARTERY REACTIVITY CHANGES IN A RABBIT MODEL OF INTIMAL SMOOTH MUSCLE PROLIFERATION

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Episodes of myocardial ischaemia at rest are consistently associated with a localized region of severe reversible epicardial vessel narrowing, as indicated by angiography (Maseri, 1987). The region of narrowing is most commonly associated with atherosclerotic involvement (Maseri, 1987). Human and animal models of atherosclerosis show evidence of smooth muscle replication, migration into the intima and further replication (Raines & Ross, 1993). We have studied carotid artery reactivity in a rabbit model of intimal smooth muscle proliferation, which does not rely on endothelial denudation.

An intimal lesion was provoked in the right carotid artery of rabbits by encasing the vessel in a silastic collar for seven days (Booth et al, 1989). The collar was then removed and the vessel allowed to recover for periods of up to 12 weeks. In another group of animals the collar was left in place for the same lengths of time. The left carotid artery was unoperated and served as a control. At each of the time points the animal was killed, the carotid arteries removed and 3mm ring sections suspended in Krebs solution at 37°C between two stainless steel hooks for the measurement of isometric tension. Resting tension was adjusted according to the length/tension relationship of each tissue. Cumulative dose-response curves for histamine, 5-hydroxytryptamine (5HT) and endothelin were constructed and responses to a single 10^{-7}M concentration of leukotriene C_4 (LTC_4) were also recorded. Tissues were fixed in formalin, sectioned and stained with haematoxylin and eosin. Responses to control and collared tissues were compared by Student's t test for unpaired data.

Endothelial-dependent responses to 10^{-6}M acetylcholine were preserved in all tissues. Responses to histamine, 5HT and endothelin were initially reduced in both collared and recovery tissues at the early time points. Maximum responses in collared vessels were 11±5%, 15±8% and 14±6% (0.01 for all) of the maximum response of the naive control, and in recovery vessels were 4±0%, 10±2% and 4±2% (0.05 for all) for histamine, 5HT and endothelin respectively at 1 week (mean±s.e.mean, p values in brackets). Reactivity increased slowly in the collared arteries so that at two weeks maximum responses were 15±4%, 21±6% and 13±5% (0.001 for all), at eight weeks were 17±5%, 24±6% and 23±7% (0.001 for all) and at twelve weeks were 35±10% (0.001), 56±16% (0.06) and 84±18% (0.7) of maximum control response for histamine, 5HT and endothelin respectively. In the recovery arteries reactivity rose rapidly at two weeks (32±7% (0.001), 62±13% (0.05) and 58±12% (0.06) for histamine, 5HT and endothelin respectively). Thereafter reactivity rose more slowly so that at eight weeks responses were 68±17% (0.04), 71±15% (0.2) and 72±13% (0.12) and at twelve weeks were 75±6% (0.01), 95±12% (0.9) and 102±12% (0.9) for histamine, 5HT and endothelin respectively. Control rabbit carotid arteries were unreactive to LTC_4, whereas 44% of collared and recovery vessels did contract, giving between 4 and 24% of the response to 80mM K^+.

Histologically the vessels showed the development of an intimal smooth muscle lesion after one week of collaring. In both collared and recovery arteries, when reactivity recovered the lesion was still present, whilst the tunica media showed a marked loss of smooth muscle cells. The results suggest that in this model, after the stimulation of intimal smooth muscle proliferation, contractile activity can recover even in the presence of an intimal lesion.

We thank the British Heart Foundation for funding.

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199P THE EFFECTS OF VERATRIDINE AND BDF 9148 ON THE CONTRACTILE FORCE OF THE RAT ISOLATED PORTAL VEIN

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Veratridine and BDF 9148 both prolong the inactivation of cardiac sodium channels to produce a positive inotropic effect (Honerjager, 1982; Brasch & Iven, 1991). Vascular smooth muscle also contains sodium channels (e.g. Mironneau et al., 1990) and we have now studied the effects of veratridine and BDF 9148 on the contractile force of the rat isolated portal vein. The method used was that of Doggrell, 1990: each group contained data from four or more portal veins and differences were analysed by student's t-test.

Veratridine at low concentrations (10^{-7} - 10^{-6}M) caused a small attenuation (10-20%; $p<0.05$), at 3 x 10^{-6}M had no effect and at 10^{-5} - 10^{-4}M augmented the force of contractions by 85-140% ($p<0.05$). BDF 9148 at 3 x 10^{-7} - 3 x 10^{-4}M attenuated the force of portal vein contractions by 10-75% ($p<0.05$). Lignocaine at 6 x 10^{-6}M had no effect on the force but prolonged the contractions of the rat portal vein. Tetrodotoxin at 10^{-7} - 3 x 10^{-5}M had no effect on the force of the spontaneous contractile activity of the portal vein. Nerve stimulation prolonged the contractions and this was abolished by tetrodotoxin at 3 x 10^{-8}M. Bay K 8644 and nifedipine at 10^{-8} - 10^{-5}M, respectively, augmented (100-500%; $p<0.05$) and attenuated (20-100%; $p<0.05$) the force responses. Lignocaine at 6 x 10^{-4}M abolished the ability of low concentrations of veratridine to attenuate the force ($p<0.05$) but not the ability of high concentrations of veratridine to cause effects (NS). Tetrodotoxin at 3 x 10^{-8} or 3 x 10^{-7}M also abolished the attenuating effects of low concentrations of veratridine ($p<0.05$). However only tetrodotoxin at 3 x 10^{-7}M abolished the augmenting actions of veratridine ($p<0.05$). The ability of BDF 9148 to alter the contractile force was only abolished in the presence of both lignocaine or tetrodotoxin and Bay K 8644. Thus in the presence of Bay K 8644 alone at 10^{-5}M high concentrations of BDF 9148 augmented the force responses by 40-160% ($p<0.05$) and this augmentation was inhibited by tetrodotoxin ($p<0.05$). In summary, veratridine at low concentrations attenuates and at higher concentrations augments the force of the rat portal vein and both effects probably involve sodium channels. BDF 9148 produces complex effects: alone it attenuates the force responses probably by blocking calcium channels but in the presence of Bay K 8644, it causes some augmentation possibly by opening sodium channels.

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200P DIFFERENCES IN BLOOD PRESSURE, HEART RATE AND VASCULAR REACTIVITY IN THE OBESE AND LEAN ZUCKER RAT

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The obese Zucker rat, an insulin resistant (IR) model, has been reported (Kurtz et al., 1989) to have elevated blood pressure compared to lean rats, though other workers have failed to observe this difference (Powloski et al., 1992). The magnitude also varies due to age differences and the methods used to measure it. We have studied these differences utilising the precise and continuous measurement of blood pressure by radiotelemetry in rats in the unstressed state over extended periods e.g. up to 3 months. An increase in vascular reactivity has been reported in-vitro in isolated aorta from obese Zucker rats compared with tissue from lean counterparts (Cox and Kikta, 1992). We have studied the blood pressure in these two phenotypes and related this to vascular reactivity changes to phenylephrine in pithed animals.

Age-matched male rats (16 - 18 wks) weighing 372 ± 10.9g (lean) or 578 ± 11.6g (obese) were cannulated via. the abdominal aorta under halothane anaesthesia for implantation of a pressure transducer (Data Sciences Inc.) and allowed to recover for 10 days. Telemetered blood pressure, expressed as mean arterial pressure (MAP) and heart rate data were collected continuously and expressed as 12 hr day and night time means. Separate groups of rats were pithed under Saffan anaesthesia and immediately ventilated with 40% oxygen. A carotid artery and jugular vein was cannulated for blood pressure measurement and drug administration respectively. Pressor response curves to phenylephrine (1 to 100ug/kg i.v.) were recorded in groups of male or female rats.

In telemetered rats daytime basal blood pressure was 121.5 ± 1.9 mmHg (n=7) in the obese rats compared with 100.5 ± 1.47 mmHg (n=10, $P< 0.001$) in the lean phenotypes. Heart rate was higher in lean (341 ± 5.23 b/min) than in obese animals (307 ± 5.2 b/min). These differences were seen in the night time readings also. Plasma levels of glucose and insulin (by radioimmunoassay) measured in tail vein blood were 6.43 ± 0.38 mM and 8.85 ± 1.10 ng/ml respectively in the obese (n=4) compared to 6.16 ± 0.40 mM and 1.93 ± 0.50 ng/ml (n=5, $P< 0.001$) in the lean. Pithed male and female obese Zucker rats demonstrated an enhanced pressor dose response to phenylephrine, e.g. in males at 30ug/kg; 109 ± 4.6 mmHg vs. 75 ± 10.6 mmHg, when this was compared with the response in lean rats (n=8, $P< 0.05$).

Thus the obese Zucker rat was hypertensive relative to its lean counterparts and demonstrated enhanced vascular reactivity to phenylephrine. In the obese rats hyperinsulinaemia was associated with the higher MAP than in lean rats with normal insulin levels. Whilst having higher MAP, the obese Zucker rat has a consistently lower heart rate, possibly indicating altered baroreflex control. The mechanisms by which IR leads to increased blood pressure are not fully understood, reactivity changes linked to decreased insulin sensitivity, when taking place alongside other changes e.g. of central nervous system and kidney function, may form the basis for a hypertensive change in the long term.

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201P DIFFERENTIAL CHANGES IN REGIONAL HAEMODYNAMICS, AND IN RESPONSE TO ACETYLCHOLINE AND METHOXAMINE, DURING LIPOPOLYSACCHARIDE INFUSION IN CONSCIOUS RATS

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Infusion of a low dose of lipopolysaccharide (LPS) inhibits carotid vasoconstrictor responses to methoxamine (ME) but generally increases vasodilator effects of acetylcholine (ACh) in conscious rats (Waller *et al.*, 1993). As a continuation of this work, we monitored the renal, mesenteric and hindquarters haemodynamic responses to ACh and ME during LPS infusion.

Male Long Evans rats were chronically instrumented with renal, mesenteric, and hindquarters pulsed Doppler probes and intravascular catheters, (under sodium methohexitone anaesthesia, 40-60 mg kg^{-1} i.p.). Catheters were implanted 7-14 days after flow probe implantation, and at least 24h before experiments were begun. Cardiovascular variables were measured during 3 min infusions (i.v.) of ACh (4 μg min^{-1}) and ME (30 μg min^{-1}), before and during the infusion of LPS (*E. coli* serotype 0127:B8 (Sigma))(150 μg kg^{-1} h^{-1}) or saline (0.3 ml h^{-1}). Saline infusion caused no marked changes in either baseline cardiovascular status or in the responses to ACh or ME. Table1 summarises the effects of LPS infusion on resting cardiovascular variables and on the responses to ACh infusion.

Table 1. Resting values (for heart rate (HR), mean arterial pressure (MAP), renal vascular conductance (RVC), mesenteric vascular conductance (MVC), and hindquarters vascular conductance (HVC)), and responses to ACh (areas under or over curves AUC, $AOC_{0\text{-}3min}$), before and during LPS infusion in conscious Long Evans rats (n=8).Values are mean ± s.e. mean, *P<0.05 versus control (Friedman's test).

	Resting Values					ACh response				
	HR (beats min^{-1})	MAP (mm Hg)	RVC ([kHz mm Hg^{-1}]10^3)	MVC	HVC	ΔHR (beats)	ΔMAP (mm Hg min)	ΔRVC ([kHz mm Hg^{-1}]10^3 min)	ΔMVC	ΔHVC
Control	333 ± 9	103 ± 1	61 ± 6	66 ± 7	53 ± 5	104 ± 9	-18 ± 4	48 ± 8	-8 ± 3	-7 ± 5
LPS 2h	377 ± 11*	90 ± 4*	106 ± 8*	59 ± 8	65 ± 9*	74 ± 18	-19 ± 3	12 ± 7*	11 ± 4*	-18 ± 6*
LPS 6h	365 ±12*	99 ± 2	88 ± 4*	68 ± 8	42 ± 3*	75 ± 8*	-9 ± 3*	25 ± 5*	17 ± 5*	-7 ± 4
LPS 24h	449 ± 8*	84 ± 2*	136 ± 10*	81 ± 7*	97 ± 8*	51 ± 14*	-39 ± 7*	50 ± 10	13 ± 7*	-18 ± 7*

After 2h of LPS infusion, there was a modest fall in MAP associated with a rise in RVC and HVC; the renal vasodilator response to ACh was abolished. The mesenteric and hindquarters vasoconstrictor, and pressor, responses to ME were reduced (ΔMAP, 83 ± 8 to 38 ± 4 mmHg min; ΔMVC, -90 ± 17 to -51 ± 8; ΔHVC, -55 ± 11 to -28 ± 7 [kHz mm Hg^{-1}]10^3 min (P<0.05)) whereas the renal vasoconstrictor responses were unchanged (ΔRVC, -57 ± 13 to -45 ± 12 [kHz mm Hg^{-1}]10^3 min). After 6h of LPS infusion, MAP was normal , RVC was still elevated, and HVC was reduced; depressor and renal vasodilator responses to ACh were reduced; pressor and vasoconstrictor responses responses to ME were all reduced (ΔMAP, 26 ± 4 mmHg min; ΔRVC, -21 ± 9; ΔMVC, -62 ± 11; ΔHVC, -9 ± 3 [kHz mm Hg^{-1}]10^3 min (P<0.05)). After 24h of LPS infusion there was hypotension associated with vasodilatation in all three vascular beds; depressor responses to ACh were enhanced, although the renal vasodilator response was not. The pressor and mesenteric vasoconstrictor responses to ME were reduced (ΔMAP, 18 ± 2 mmHg min; ΔMVC, -32 ± 7 [kHz mm Hg^{-1}]10^3 min (P<0.05)), whereas renal and hindquarters vasoconstrictor responses were not (ΔRVC, -35 ± 8; ΔHVC, -33 ± 5 [kHz mm Hg^{-1}]10^3 min).

The present findings, together with our previous observations, could indicate regional heterogeneity in the processes activated by LPS, which may change with time.

J.W. is supported by an MRC studentship.

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202P RELAXATION RESPONSES OF RAT BLOOD VESSELS TO PIMOBENDAN AND ITS MAJOR ACTIVE METABOLITE UD-CG 212

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(Introduced by K.F.Ilett).

Pimobendan, a novel inotropic agent, has also been shown to possess arteriodilator properties (Duncker et al., 1987). The vasodilating effect of pimobendan was partly due to an increase in cyclic AMP resulting from phosphodiesterase (PDE) inhibition (Fujimoto and Matsuda, 1990), to the blockade of α-adrenoceptor-operated Ca^{2+} channels, and due to an intracellular action to reduce cytoplasmic free Ca^{2+} levels (Delgado et al., 1987). Pimobendan is demethylated in the body to UD-CG 212, 2-(4-hydroxy-phenyl)-5-(5-methyl-3-oxo-4,5-dihydro-2H-6-pyridazinyl) benzimidazole, which can elicit a positive inotropic and vasodilating effect, partly via the inhibition of cyclic AMP PDE (Delgado et al., 1987). In the present study, vascular actions of pimobendan and UD-CG 212 were compared with those of milrinone, another cyclic AMP PDE inhibitor. Helical strips of rat blood vessels were suspended under optimal resting tensions (aorta 1 g, femoral 500 mg, carotid 400 mg and mesenteric 100 mg) in Krebs solution. Pimobendan, UD-CG 212 and milrinone relaxed the vascular strips (contracted with K^+ 30mM) in a dose-dependent manner. UD-CG 212 was more potent as a vasorelaxant than pimobendan in the aorta and carotid artery, but was as potent as pimobendan in the femoral artery and mesenteric vein (Table 1). Milrinone was less potent than pimobendan in femoral artery and mesenteric vein. These drugs potentiated isoproterenol- but not glyceryl trinitrate-induced relaxations, and reduced Ca^{2+}-induced contractions of the femoral artery in a non-competitive manner. It is suggested that the relaxant responses to these drugs are explained by their inhibitory effects on cyclic AMP PDE activities.

Table 1. Negative log EC_{50} values for pimobendan-, UD-CG 212-, and milrinone-induced relaxations

	Thoracic aorta	Femoral artery	Carotid artery	Mesenteric vein
Pimobendan	5.25±0.04	$6.37±0.05^{a}$	$5.91±0.08^{abc}$	$6.45±0.10^{a}$
UD-CG 212	$5.64±0.07^{d}$	$6.86±0.22^{ab}$	$6.88±0.07^{abd}$	$6.23±0.13^{a}$
Milrinone	5.05±0.08	$5.72±0.11^{ad}$	$5.73±0.09^{a}$	$5.52±0.15^{ad}$

Data are expressed as means±s.e.mean (n=6-8). An analysis of variance with Bonferroni t-test was used for the statistical analysis. P<0.05 compared with aorta[a], mesenteric vein[b], femoral artery[c], and with pimobendan[d].

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203P CHARACTERISATION OF CAPSAICIN-INDUCED VASODILATION OF THE PERFUSED RABBIT EAR AND ITS MODULATION BY DEXAMETHASONE

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Topical glucocorticoids inhibit the vasodilation caused by local non-pathological heating of the skin (Ahluwalia & Flower, 1993). This response is at least in part mediated by the activation of cutaneous sensory afferent neurones suggesting an inhibitory effect of the topical steroids at the sensory neurone. To investigate further this possibility we have used the perfused rabbit ear preparation to provide a convenient system in which the steroid effects upon skin microcirculation may be examined.

Male New Zealand White rabbits (2.6-3.0 kg) were anaesthetised with sodium pentobarbitone (30 mg/kg, i.v.) and the central ear artery cannulated in both ears. After sacrifice the ears were removed at the base and perfused with Krebs' solution (36°C) gassed with 5 % CO_2 in O_2 at a rate of 2 ml/min. Basal perfusion pressure after 1h equilibration measured 39.1 ± 4.1 mmHg (n=36). A predetermined EC50 concentration of phenylephrine (5-10 nmol/min) was used to induce tone. Dose-response curves to capsaicin (0.001-100 nmol) were constructed in each ear, one of which was perfused with putative antagonists 30 min prior to capsaicin while the other ear remained as an untreated control. To investigate the effect of glucocorticoids upon the vasodilator response dexamethasone (DEX, 2.6 μM) or vehicle was perfused into the ears for 1h after the first dose-response curve to capsaicin and a second curve constructed after 1 h.

The responses to capsaicin were blocked significantly ($p < 0.01$, n=3) by the calcitonin gene related peptide (CGRP) receptor antagonist $CGRP_{8-37}$ (1 μM) and the vanilloid receptor antagonist, capsazepine (10 μM), caused a rightward shift (~12-fold; $p < 0.05$, n=4), while the tachykinin NK-1 receptor blocker, CP96345 (100 nM) had no significant effects (n=4). Finally DEX treatment had no effect upon the responses to CGRP (0.26-2600 pmol, n=4) or capsaicin. In untreated ears the second dose-response curve to capsaicin was significantly ($p < 0.05$, n=6) shifted to the right together with a depression of the maximum. There were no significant differences between the responses to capsaicin after treatment with DEX. Radioimmunoassy of effluent samples showed CGRP leakage occurring during the 1h interval between curves to capsaicin (276.4 ±29.0 % of release occuring during construction of control capsaicin curve), and this release was found to be significantly (190.4 ± 10.9 %, $p < 0.05$, n=4) reduced in DEX-treated ears.

In this preparation capsaicin acts through the vanilloid receptor releasing CGRP which produces vasodilation. Since DEX has no effect upon exogenous CGRP action, it must act at a pre-junctional level, perhaps by affecting the chronic release of neuropeptides reported to occur after capsaicin treatment (Huang *et al.*, 1993) which could lead to depletion of these transmitters.

AA is supported by Wellcome and RJF by an endowment from Lilly, UK. We would like to thank The Sandoz Institute for Medical Research, UK for the supply of capsazepine, Pfizer, USA for CP96345 and Paul Newbold for carrying out the radioimmunoassay.

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204P DO ENDOTHELIUM-DERIVED PROSTAGLANDINS AFFECT α_2-ADRENOCEPTOR-MEDIATED Ca^{2+} ELEVATIONS IN CULTURED AORTIC SMOOTH MUSCLE CELLS?

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In cultured porcine aortic smooth muscle cells (PASMC) α_2-adrenoceptor stimulation (e.g. by α-methyl-noradrenaline) increases intracellular free Ca^{2+} (Erdbrügger et al. 1993). In vivo hormonally-stimulated vasoconstriction is modulated by endothelium-derived prostaglandins (PGs; Furchgott & Vanhoutte, 1989). Therefore, we have compared the pattern of PG formation in PASMC and cultured porcine endothelial cells (EC), and investigated whether PGs affect α_2-adrenoceptor-mediated Ca^{2+} elevations. Cultured EC and PASMC were labelled overnight with 10 µCi [^{3}H]arachidonic acid and stimulated mechanically by a rubber policeman or chemically with the Ca^{2+}-ionophore A23187 (10 µM) for 20 min at 37°C in 10 mM HEPES, 150 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$ and 5 mM glucose (pH 7.5). [^{3}H]PGs were extracted using C_{18}-columns, separated on silica plates (water saturated ethylacetate/isopropanol/acetic acid 75/30/15 v/v/v) and were visualized by autoradiography. Intracellular Ca^{2+} was measured using fura-2 (Erdbrügger et al., 1993).

EC produced similar amounts of total PGs following mechanical and chemical stimulation but PGI_2 was the main product upon mechanical (56 ± 5% of total PGs; n=3) whereas $PGF_{2\alpha}$ dominated upon chemical stimulation (59 ± 7%); the formation of PGE_2 was similar after both stimuli (≈ 10-15%). PASMC formed considerably less total PGs than EC and PGE_2 was the main product with either stimulus (≈80% of total PGs). PGs formed by PASMC are unlikely to modulate the Ca^{2+} response to α_2-adrenoceptor stimulation since preincubation with indomethacin, nordihydroguaretic acid (1 µM each) or both had no effect. Arachidonic acid (20 µM) and iloprost (10 µM) did not modulate the Ca^{2+} response to α-methyl-noradrenaline whereas 10 µM PGE_2 inhibited it by 30 ± 6% (n=8). $PGF_{2\alpha}$ (10 µM) itself increased intracellular Ca^{2+} by 30 ± 12 nM (n=3) but did not affect the response to α-methyl-noradrenaline. We conclude that porcine aortic EC secrete different patterns of PGs upon mechanical and chemical stimulation. Among the PGs formed by EC only PGE_2 modulates α_2-adrenergic Ca^{2+} elevations. Since PGE_2 is the least abundant PG formed by EC, we conclude that this may not be of major physiological relevance.

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205P α_1-ADRENOCEPTOR BINDING SITES IN THE EPIDIDYMAL AND PROSTATIC RAT VAS DEFERENS

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Recent evidence has suggested there are differences in α_1-adrenoceptor populations between the two ends of the rat vas deferens. For example, using [^{3}H]-prazosin, the epididymal portion of the vas deferens was found to contain high and low affinity binding sites while the prostatic portion had only a single site (Ohmura *et al*, 1992). In whole vas deferens, Minneman *et al* (1988) showed the existence of WB-4101 low and high affinity binding sites. In the present work, [^{3}H]-prazosin binding sites were examined in the epididymal and prostatic portions of the rat vas deferens using the α_{1A}-selective antagonists WB-4101, 5-methyl-urapidil and (+)-niguldipine, and compared to binding in rat α_{1A}-(brain pretreated with chlorethylclonidine, CEC) and α_{1B}-(liver) binding sites.

Membrane homogenates were prepared in ice-cold 50mM Tris buffer, pH 7.4 from brain (cerebellum removed), liver and epididymal and prostatic vas deferens (from Sprague Dawley rats). Competition assays were conducted at 25°C for 30min with 0.5mg/ml membrane protein, 0.4nM [^{3}H]-prazosin and competing drug in a final volume of 0.5ml. Non-specific binding was determined in the presence of 10μM phentolamine. Saturation assays were performed in the presence of buffer or phentolamine over a range of [^{3}H]-prazosin concentrations. CEC pretreatments were performed at 37°C in 10mM Tris HCl buffer, pH 7.4 for 15min. The pretreatments were terminated with ice-cold 50mM Tris buffer followed by three washes. Data are given as the mean±s.e.mean of between 3 and 10 experiments unless indicated otherwise. In the saturation experiments [^{3}H]-prazosin bound with a single affinity in all tissues. In brain, CEC pretreatment reduced the B_{max} from 214.0±12.2 to 116.9(n=2) fmol/mg protein (K_D=0.26±0.03 and 0.54 nM respectively). In liver, CEC pretreatment reduced the B_{max} from 206.4±21.6 to 52.9±6.0 fmol/mg protein (K_D=0.20±0.04 and 0.28±0.03 nM respectively). Epididymal and prostatic portions of the vas deferens had B_{max} values of 153.9(n=2) and 351.5±11.7 fmol/mg protein and K_D values of 0.6 and 0.3±0.02 nM respectively. In competition assays (Table1), prazosin was equipotent in all tissues. WB-4101, 5-methyl-urapidil and (+)-niguldipine showed 20-,10- and 7-fold selectivities respectively for α_{1A} (CEC-pretreated brain) over α_{1B} (liver) . WB-4101 revealed high affinity binding sites in both ends of the vas deferens. 5-methyl-urapidil showed high affinity binding in the epididymal portion but a lower affinity in the prostatic portion whereas (+)-niguldipine showed intermediate affinity in both portions. These results indicate that under conditions which allow α_{1A} and α_{1B} binding sites in rat tissues to be distinguished, there are no apparent consistent differences between epididymal and prostatic portions of the rat vas deferens. The shallow 'Hill' slopes observed for the vas in this study are similar to the results of Ohmura *et al* (1992) and together with the K_i values obtained do not rule out the possibility of heterogeneity of α_1-adrenoceptor subtypes in both ends of the rat vas deferens.

Table 1

Tissue	K_i,nM(slope)			
	Prazosin	WB-4101	5-Methyl-urapidil	(+)-Niguldipine
Brain	0.1±0.02 (0.9±0.03)	1.8±0.4 (0.6±0.05)	12.7±3.6 (0.6±0.05)	33.7±9.9 (0.7±0.03)
Brain + CEC	0.3±0.08 (0.8±0.04)	0.3±0.05 (0.6±0.03)	3.2±0.5 (1.0±0.08)	29.5±5.5 (0.9±0.05)
Liver	0.1±0.04 (0.9±0.07)	6.4±0.09 (0.8±0.06)	31.2±3.3 (0.9±0.1)	204.7±26.6 (0.9±0.1)
Epididymal vas deferens	0.3±0.1 (0.5±0.04)	0.7±0.01 (0.5±0.01)	4.3±0.2 (0.7±0.1)	47.3±24.0 (0.6±0.2)
Prostatic vas deferens	0.2±0.02 (0.7±0.1)	0.5±0.1 (0.6±0.09)	10.1±1.5 (0.6±0.04)	61.1±26.7 (0.5±0.04)

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206P ENDOTOXIN IMPAIRS THE RESPONSE TO NERVE STIMULATION IN THE RAT ISOLATED PERFUSED MESENTERIC BED

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Endotoxin decreases vascular responsiveness to α-adrenoceptor agonists in vivo (Guc *et al.*, 1990) and in blood vessels isolated from endotoxin-treated rats (Bigaud *et al.*, 1990). Endotoxin also impairs pressor responses to sympathetic nerve stimulation in the pithed rat (Gray *et. al.*, 1990). This might be explained by a decreased response to released noradrenaline although inhibition of neurotransmitter release may also contribute.

In the present work we have examined the effect of endotoxin on pressor responses to noradrenaline and to electrical stimulation of the rat isolated, perfused mesenteric bed. Rats were anaesthetised with pentobarbitone (60 mg kg^{-1} i.p.) and ventilated artificially (54 strokes / min, 1.5 ml / 100g). After a 4h infusion of either saline or *E. coli* endotoxin (Difco 055: B5, 2.5 mg kg^{-1} h^{-1}), mesenteric beds were removed and perfused with Krebs buffer (95 % O_2/5% CO_2; 37°C, 2 ml min^{-1}, constant flow, 20 mmHg initial pressure) via the mesenteric artery. After 20 min equilibration, electrical stimulation was started (supramaximal voltage, 3 ms duration, 7-40 Hz every 15 min) using electrodes around the mesenteric artery. There was a frequency-dependent increase in perfusion pressure in response to nerve stimulation in the mesenteric beds, which was completely blocked by prazosin (2 x 10^{-7} M) or guanethidine (2-4 x10^{-7} M). Preparations from endotoxin-treated rats showed significantly reduced responses at all stimulation frequencies (eg., increase in perfusion pressure (mmHg) at 10 Hz; control 43.1 ± 5.2, endotoxin 14.7 ± 3.9, n = 7, P <0.05). However, endotoxin treatment did not modify responsiveness to noradrenaline (10^{-7} - 10^{-5} M). L-NAME (3 x 10^{-3} M) appeared to restore responses to nerve stimulation in preparations from endotoxin-treated rats but also augmented such responses in control preparations. Two way ANOVA suggested that the effect of L-NAME was the same in both types of preparations (Increase in perfusion pressure, mmHg at 10 Hz; control 43.1 ± 5.2, L-NAME & control 54.7 ± 1.2, endotoxin 14.7 ± 3.9, endotoxin & L-NAME 45 ± 1.2; effect of endotoxin P < 0.01; effect of L-NAME P < 0.01, interaction NS). Responses to NA were also augmented by L-NAME (eg. increase in perfusion pressure to NA, 4 x 10^{-6} M, mmHg ; control 9 ± 0.6, L-NAME 42 ± 1.5, P = 0.002). These data suggest that endotoxin-induced impairment of responsiveness to nerve stimulation in rat mesenteric beds may be a prejunctional effect. The observation that the effect of L-NAME was similar in preparations from control and endotoxin treated rats does not support a role for the L-arginine-nitric oxide pathway in this impairment, although this requires further study.

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207P CHARACTERISATION OF THE α_1-ADRENOCEPTOR MEDIATING VASOCONSTRICTOR RESPONSES TO NORADRENALINE AND NERVE STIMULATION IN RAT ISOLATED PERFUSED MESENTERY

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α_1-Adrenoceptors have been sub-divided operationally into α_{1A}-, α_{1B}- and α_{1L}-subtypes; other sub-divisions have been made from molecular cloning studies (for references see Muramatsu, 1992). The present studies have investigated the relative contribution of α_1-adrenoceptor subtypes to noradrenaline (NA)-induced vasoconstrictor responses in an isolated perfused mesentery preparation from rat.

Isolated mesentery preparations (with intestine attached) from male Sprague-Dawley rats were perfused via the superior mesenteric artery with Krebs' solution (37°C, pH 7.4) at a rate of 6ml min^{-1}. The perfusate was of the following composition (mM); Na^+ 143.5, K^+ 6.0, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 128.3, HCO_3^- 25, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2, glucose 11.0, ascorbate 0.1, cocaine 0.03, corticosterone 0.03, indomethacin 0.01, propranolol 0.001 and idazoxan 0.0003. Studies assessed the effects of perfused antagonists on vasoconstrictor responses to either perfused NA (non-cumulative concentration-response curves) or periarterial electrical stimulation (frequency-response curves; 0.3Hz-100Hz, 70V, 2ms pulse width, 10s train). Cocaine and corticosterone were not used for the electrical nerve stimulation studies.

Perfused NA caused a concentration-related increase in perfusion pressure (EC_{50} approximately 3-10μM) which was antagonised competitively by prazosin, WB4101, 5-methylurapidil (5-MU) and spiperone, with respective pA_2 estimates of 9.3±0.1, 9.6±0.1, 9.0±0.1 and 7.7±0.1 (mean ± s.e.mean, n=3-6). Chloroethylclonidine (CEC), an irreversible α_{1B}-adrenoceptor antagonist (100μM for 20min followed by a 40min washout period) caused a two-fold dextral shift in the concentration-response curve to perfused NE, but did not affect significantly pA_2 estimates (mean ± s.e.mean, n=3) for prazosin (9.2±0.1), WB4101 (9.3±0.1) or 5MU (8.7±0.2). Responses to electrical stimulation (measured as the area under the frequency-response curve) were antagonised by prazosin, WB4101, 5-MU and spiperone with respective IC_{50} values of 1.3nM, 0.5nM, 1.0nM and 38nM. These IC_{50} values correlated well with the derived pA_2 values (see above). Following exposure to CEC at 10μM and 100μM (both for 20min with a 40min washout), responses to electrical stimulation were 79±7% (n=12) and 46±5% (n=7) of control respectively (mean ± s.e.mean).

The potent antagonism of vasoconstrictor responses to either perfused or neuronally-released NA by the α_{1A}/α_{1C}-adrenoceptor-selective antagonists WB4101 and 5-MU, and the relatively weak effect of the α_{1B}-adrenoceptor-selective antagonist spiperone suggest that these responses are mediated via α_{1A}- or α_{1C}-adrenoceptors. Since the operationally-defined α_{1A}-adrenoceptor is considered to be insensitive to CEC, the receptor characterised in the present study may represent a rat homologue of the cloned bovine α_{1C}-adrenoceptor, which has been shown to be CEC-sensitive, and to exhibit high affinity for WB4101 and 5-MU.

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208P AFFINITY AND EFFICACY OF SDZ NVI-085 AT RAT α_1-ADRENOCEPTOR SUBTYPES

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SDZ NVI-085 ((-)-(4aR,10aR)-3,4,4a,5,10,10a-hexahydro-6-methoxy-4-methyl-9-methylthio-2H-naphth[2,3,b]-1,4-oxazine HCl) is a novel anticataplectic agent (Renaud et al. 1991). We have investigated its affinity at α_1-adrenoceptors in rat spleen, cerebral cortex, and kidney (containing only α_{1B}, mixed α_{1A}/α_{1B}, and mixed α_{1A}/α_{1B} and possibly a third subtype, respectively [Michel et al. 1993]) and its effects on inositol phosphate formation in rat renal cortex.

α_1-Adrenoceptor affinities were determined in [^{3}H]prazosin competition binding studies and effects on inositol phosphate formation in renal cortical slices during a 45 min incubation at 37° C as previously described (Michel et al. 1993). In some cases renal α_{1B}-adrenoceptors were inactivated by treatment with 10 μM chloroethylclonidine for 30 min at 37° C. Data are mean ± s.e.mean of 3-5 experiments. In rat spleen SDZ NVI-085 competed for [^{3}H]prazosin binding with steep curves (pK_i of 4.79±0.05). Competition curves in cerebral cortex were also quite steep (nH 0.86±0.04; pK_i 4.83±0.02) and biphasic analysis was not consistently possible. In rat kidney SDZ NVI-085 competition curves were shallow (nH 0.67±0.05) and biphasic with 31±4% high affinity sites ($pK_{i\ high}$: 6.50±0.28, $pK_{i\ low}$: 4.75±0.20). Similar data were obtained in the presence of 100 μM GTP. Following chloroethylclonidine treatment SDZ NVI-085 competition curves in renal membranes were steepened (nH 0.87±0.02; pK_i: 5.51±0.33). In renal cortical slices 0.1 and 1 mM of SDZ NVI-085 enhanced inositol phosphate formation only weakly (≈35% of the maximal response to noradrenaline) with lower concentrations being without effect.Accordingly 1 mM SDZ NVI-085 inhibited the response to 0.1 mM noradrenaline by 74%. This was similarly observed in native and in chloroethylclonidine-treated slices.

We conclude that SDZ NVI-085 is a partial agonist at α_1-adrenoceptors. It may be selective for some type of chloroethylclonidine-insensitive α_1-adrenoceptor but this selectivity is not readily explained by the α_{1A}/α_{1B}-adrenoceptor concept. Affinities of SDZ NVI-085 at cloned α_1-adrenoceptor subtypes will be communicated separately (Michel & Insel, 1994).

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209P ISOPRENALINE RELAXES RAT ISOLATED MESENTERIC ARTERY RINGS VIA ENDOTHELIUM-DEPENDENT AND INDEPENDENT MECHANISMS

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The relaxation of vascular smooth muscle by β–adrenoceptor agonists in many instances is thought to be independent of the endothelium (Furchgott, 1984), although there is recent evidence for an endothelium-dependent β–adrenoceptor mediated relaxation in the rat thoracic aorta and in rat hindquarters *in vivo* (Gray and Marshall, 1992; Gardiner *et al.*, 1991). In the present experiments the mechanism of relaxation to isoprenaline in the rat mesenteric artery has been investigated.

Rat mesenteric arterial rings were suspended in Krebs solution at 37°C and oxygenated with 95%O_2/5%CO_2 for isometric tension recording. Arteries were preconstricted with noradrenaline ($3x10^{-7}$M) and a cumulative concentration curve to isoprenaline constructed. In some tissues the endothelium was removed by gentle abrasion of the intima which abolished the relaxation to acetylcholine (10^{-6}M). Other tissues were pretreated with propranolol (10^{-7}M), N^G-nitro-L-arginine (L-NOARG;10^{-6}M and 10^{-5}M), methylene blue (10^{-5}M) or calmidazolium (Weinheimer and Osswald, 1986) (10^{-5}M), for at least 20 min before a second dose-response curve to isoprenaline was obtained.

Isoprenaline (10^{-8}M-10^{-4}M) caused a concentration-dependent relaxation of noradrenaline tone in mesenteric arterial rings with intact endothelium (pD_2 6.7±0.2; mean±s.e.mean; max. relax.=100%). Propranolol (10^{-7}M; pK_B=8.2) caused a parallel rightward shift in the isoprenaline dose-response curve with no reduction in maximum response. Removal of the endothelium shifted the isoprenaline curve to the right and decreased the maximum to 62±7% (pD_2 4.7±0.1). In intact rings L-NOARG (10^{-6}M and 10^{-5}M) caused a rightward shift of the isoprenaline curve by 19- and 79-fold, respectively. The inhibition of relaxation by L-NOARG (10^{-6}M) was stereospecifically reversed by L-arginine (10^{-4}M) but not D-arginine (10^{-4}M). Pre-incubation with L-NOARG (10^{-5}M) or removal of the endothelium approximately doubled the contractile response to noradrenaline ($3x10^{-7}$M; control response 0.6±0.1g) in mesenteric arterial rings ($p<0.01$, Student's t-test). Methylene blue (10^{-5}M), a soluble guanylate cyclase inhibitor, increased the contraction to noradrenaline ($3x10^{-7}$M) and inhibited the relaxant response to isoprenaline (pD_2 4.6±0.1; max. relax.=97±1%). The calmodulin inhibitor, calmidazolium (10^{-5}M), attenuated the relaxation caused by isoprenaline (pD_2 4.7±0.1) and decreased the maximum relaxation to 80±3% without affecting the relaxation to sodium nitroprusside.

Removal of the endothelium, inhibition of nitric oxide synthase and calmidazolium reduced the β–adrenoceptor mediated relaxation of mesenteric arterial rings which suggests an endothelial-dependent mechanism. However, the persistence of relaxation after the removal of the endothelium or after L-NOARG or calmidazolium suggests an additional mechanism of relaxation and for which isoprenaline has a lower affinity. Therefore there appear to be two mechanisms underlying the β–adrenoceptor mediated relaxation of the rat mesenteric artery, endothelial-dependent and independent.

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210P CARDIAC β_1- AND β_2-ADRENOCEPTOR UPREGULATION AFTER 6-OHDA-INDUCED SYMPATHECTOMY

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Reports of changes in the binding density of cardiac β_1- and β_2-adrenoceptors after chemical sympathectomy with 6-hydroxydopamine (6-OHDA) are inconsistent (*cf* Tumer, 1989; Michel *et al.*, 1987). Possible explanations for this include the use hitherto of radioligands which are either non-β-selective or lipophilic, or both. Here, the β-selective, lipophobic radioligand, (-)-4-(3-t-butylamino-2-hydroxypropoxy)-[5,7-^{3}H]-benzimidazol-2-one ([^{3}H]CGP 12177), was used to measure changes in cardiac β_1- and β_2-adrenoceptors after 6-OHDA.

Rats were injected with either 100 mg/kg 6-OHDA (2 x 50 mg/kg i.v. separated by 24h) or vehicle. 7 or 21 days later animals were killed and cardiac noradrenaline content and β-adrenoceptor binding measured *ex vivo*. The density of total (β_1 + β_2)-adrenoceptors was measured using [^{3}H]CGP 12177 (0.05-5nM); specific binding was defined by the non-selective β-agonist, (-)-isoprenaline (500μM). In parallel aliquots of tissue, specific binding to β_1-adrenoceptors was defined by the selective β_1-antagonist, (1-[2-((3-carbamoyl-4-hydroxy)phenyoxy)ethylamino]-3-[4-(1-methyl-trifluoromethyl-2-imidazolyl)phenoxy]-2-propanol methanesulfonate (CGP 20712A). Binding to β_2-adrenoceptors was calculated by subtraction of these two sets of measurements (see Dooley & Bittiger, 1987).

Table 1 Effects of 6-OHDA on cardiac noradrenaline levels and the density of β-adrenoceptor subtypes

		NA concentration (ng/g wet weight)	β_1Bmax	(β_1+β_2) Bmax (pmol/g.protein)	β_2Bmax
7 days	Vehicle	531 ± 60	18 ± 3	20 ± 2	2.5 ± 0.6
	6-OHDA	109 ± 8 *	24 ± 2 *	28 ± 2 *	4.1 ± 0.8 *
21 days	Vehicle	356 ± 14	14 ± 2	17 ± 2	3.2 ± 0.4
	6-OHDA	107 ± 8 *	20 ± 2	23 ± 1 *	3.4 ± 0.4

Values show mean ± s.e.mean. N=7-9 for each sample. *P<0.05 *cf* corresponding vehicle.

7 days after 6-OHDA injection there was a marked (89%) depletion of noradrenaline which was paralleled by a statistically significant increase in the density of both β_1-adrenoceptors (32%) and β_2-adrenoceptors (64%). The noradrenaline concentration in lesioned hearts remained the same for a further 14 days and the increase (33%) in the density of β_1-adrenoceptors persisted. However, β_2-adrenoceptor density returned to control levels by this time. The upregulation of β_1-adrenoceptors is consistent with a response to denervation, but mechanisms underlying the transient upregulation of β_2-adrenoceptors remain to be explained.

Acknowledgements: We are indebted to Beckman Instruments (UK) Ltd, the Sutasoma Trust and the Wellcome Trust for financial support.

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211P B_1 AND B_3 ADRENOCEPTORS IN GUINEA-PIG ILEUM: BIOCHEMICAL AND FUNCTIONAL INTERACTION WITH M_2 MUSCARINIC RECEPTORS

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In guinea-pig ileum, both β_1 and β_3 adrenoceptors inhibit development of longitudinal smooth muscle tension (Bond & Clarke, 1988). Muscarinic contraction of this tissue is mediated by M_3 receptors, although 80% of muscarinic receptors are of the M_2 subtype (Ford *et al.*, 1991). Recent evidence suggests that in ileum, after M_3 receptor alkylation, M_2 receptors cause contraction by offsetting β adrenoceptor relaxation in tissues pre-contracted to histamine (Thomas *et al.*, 1993). The aim of this study is to investigate the effect of muscarinic agonism on both the biochemical and functional responses to β_1 (using isoprenaline) and β_3 (using BRL 37344) adrenoceptor stimulation.

Effects on adenylyl cyclase activity were investigated in guinea-pig ileal longitudinal smooth muscle slices labelled with [^{3}H] adenine. Accumulation of [^{3}H] cyclic AMP, in the presence of 1 mM 3-isobutyl-1-methyl xanthine and 0.1 μM forskolin, was determined as described by Alvarez & Daniels (1992). Potency and affinity values are mean ± s.e. mean. Isoprenaline and BRL 37344, in the presence of 3 μM idazoxan, increased cyclic AMP accumulation with -log EC_{50} values of 6.6 ± 0.1 (n=6) and 5.8 ± 0.1 (n=5). Maximal stimulation by BRL 37344 (10 μM) was 26 ± 5.2% of that observed with isoprenaline (10 μM). The non-selective muscarinic receptor agonist, (+)*cis*-dioxolane, inhibited basal, isoprenaline and BRL 37344 stimulated cyclic AMP accumulation. Isoprenaline (10 μM) stimulated cyclic AMP accumulation was inhibited by *(+)cis*-dioxolane with a -log EC_{50} value of 7.3 ± 0.1 (n=11). This effect was blocked by atropine, pirenzepine, methoctramine and *para*-fluoro-hexahydrosilafendidol, with -log K_B estimates of 9.0 ± 0.2, 6.3 ± 0.2, 7.1 ± 0.1, 6.5 ± 0.2, respectively (n=4-5).

To investigate M_2 receptor-mediated effects on tension development, segments of ileum were suspended under tension (5 mN) in modified Krebs' solution (pH 7.4, 37°C). Selective M_3 receptor alkylation was achieved with 4-DAMP mustard (40 nM) for 60 min in the presence of methoctramine (1 μM). After repeated washing over 100 mins, tissues were exposed to histamine (0.32 μM) and isoprenaline (0.64 μM) and cumulative concentration-response curves to oxotremorine-M were constructed. Under these conditions, oxotremorine-M caused contraction with -log EC_{50} of 7.8 ± 0.1 (n=6). Methoctramine (1μM) caused a parallel rightward shift of this curve (-log EC_{50} of 6.4 ± 0.1) yielding a -log K_B value of 7.4 ± 0.1 (n=6). The above protocol was repeated using histamine (1μM) and BRL 37344 (1 μM), in the presence of propranolol (5 μM). Oxotremorine-M again caused contraction (-log EC_{50} = 8.0 ± 0.1;n=5) and methoctramine (1 μM) antagonised this contraction (-log EC_{50} = 6.2 ± 0.1) yielding a -log K_B value of 7.8 ± 0.2 (n=5).

These data are consistent with β_1 and β_3 adrenoceptors coupling to stimulation of adenylyl cyclase in guinea-pig ileum; a response inhibited by M_2 receptor agonism. Furthermore, M_2 muscarinic receptor activation apparently inhibits relaxation to β_1 or β_3 adrenoceptor stimulation. The data presented may implicate a role for M_2 muscarinic receptors in the modulation of sympathetic control of ileal motility.

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212P (–)-[^{125}I]-CYANOPINDOLOL BINDS TO A SITE RESEMBLING ATYPICAL β-ADRENOCEPTORS IN GASTROINTESTINAL TISSUES

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Functional studies of adrenergic responses in gastrointestinal tissues have suggested a population of atypical ß-adrenoceptors (ß-AR) with characteristics resembling the $ß_3$-AR (Bond & Clarke, 1988; McLaughlin *et al.*, 1988; van der Vliet *et al.*, 1990). Identification of these sites using receptor binding assays has been hindered by the low affinity of ß-AR ligands at atypical ß-AR's and the lack of a selective atypical ß-AR antagonist. A propranolol-resistant (-)-[^{125}I]-cyanopindolol (ICYP) binding site has been described in rat soleus muscle with characteristics resembling those of the atypical ß-AR (Roberts *et al.*, 1993). The present study uses this binding assay to characterise binding sites in rat ileum (RI) and human colon (HC). Homogenates of RI and HC were prepared and aliquots incubated with ICYP at 37°C for 60 min, with (±)-alprenolol (500 μM) to define non-specific binding. Saturation studies with ICYP (5-1000pM) revealed low affinity binding sites in both tissues: K_D RI 843 ± 125pM (Bmax 50 ± 4 fmol/mg protein)(n=4); K_D HC 691 ± 385pM (Bmax 53 ±8 fmol/mg protein)(n=4). Competition for ICYP (500pM) binding showed a low affinity of propranolol for the atypical site. (Table 1). Competition experiments performed in the presence of 0.1 μM propranolol (blocks 98% $ß_1/ß_2$-AR's, 3% atypical sites) provided affinities for ß-AR antagonists (Table 1). Stereoselective competition for ICYP binding was higher in HC (alprenolol 6 fold; tertatolol 9 fold) than in RI (alprenolol 4 fold; tertatolol 2 fold). Competition for ICYP binding by atypical ß-AR agonists showed low affinities in both tissues: pK_D values: SR 58611A (HC 5.13 ± 0.07, RI 4.26 ± 0.11), ICI D7114 (HC 4.36 ±0.09, RI 4.58 ± 0.07) and BRL 37344 (HC 3.83 ± 0.07, RI 3.64 ± 0.13), all (n=4). This study provides evidence for ß-AR in HC and RI with characteristics resembling those of the atypical ß-AR's described in functional studies in gastrointestinal tissues.

Table 1. Competition studies: pK_D values (mean ± s.e.mean (n))

competitor	(-)-propranolol	(±)-CYP	(-)-alprenolol	(+)-alprenolol	(-)-tertatolol	(+)-tertatolol
pK_D (RI)	5.47 ± 0.14 (6)	6.92 ± 0.04 (5)	5.62 ± 0.07 (5)	5.05 ± 0.04 (6)	6.16 ± 0.05 (6)	5.90 ± 0.05 (6)
pK_D (HC)	5.30 ± 0.05 (4)	7.57 ± 0.04 (4)	6.29 ± 0.07 (4)	5.49 ± 0.06 (4)	7.13 ± 0.08 (4)	6.16 ± 0.06 (4)

S.J.R. is a Commonwealth Postgraduate Scholar.

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213P ZENECA ZD2079 IS A NOVEL β_3-ADRENOCEPTOR AGONIST

Grant, T.L., Mayers, R.M., Quayle, S.P., Briscoe, M.G., Howe, R., Rao, B.S., Growcott, J. and Holloway, B.R. ZENECA Pharmaceuticals, Macclesfield, Cheshire, SK10 4TG.

β_3-adrenoceptor agonists are improve insulin sensitivity and are thermogenic suggesting that they have potential in the treatment of non-insulin-dependent diabetes mellitus (NIDDM) and obesity. The aim of this paper is to describe the pharmacology of ZENECA ZD2079 ((R)-N-(2-[4-(carboxymethyl)phenoxy]ethyl)-N-(β-hydroxyphenethyl)ammonium chloride), a novel β_3-adrenoceptor agonist.

Measurement of free fatty acid levels (Quayle *et al*, 1993) revealed that ZD2079 increased lipolysis in rat epididymal white adipocytes with an EC_{50} of 0.35μM (95% confidence limits: 0.3-0.4, n=12). ZD2079 had a maximal effect (102.7±1.4%) equivalent to that evoked by both BRL 37344 (103.6± 2.0%), a standard β_3-adrenoceptor agonist, and isoprenaline (100%). ZD2079 increased oxygen consumption in conscious Wistar rats in a dose-related manner (ED_{50}= 0.41mg/kg po, 95% confidence limits: 0.32-0.51, n=3) achieving a maximal effect at 3.0 mg/kg. ZD2079 also produced a dose-related increase in rat brown adipose tissue activity as measured by specific mitochondrial GDP binding (ED_{50}=0.55mg/kg po; 95% confidence limits: 0.39-0.78, n=7). In addition, in the conscious rat, ZD2079 significantly increased heart rate (HR) at doses ≥5.24mg/kg (po)(ie at doses greater than those required for a maximal increase in metabolic rate): control : 359±9 BPM; 5.24mg/kg: 420±10 BPM; 26.2mg/kg: 437±7 BPM; 52.4mg/kg: 462±10 BPM ($p<0.001$, n= 6). All conscious rat methods were as described by Holloway *et al* (1991).The effects of ZD2079 (10μM) in guinea-pig right atria (β_1-adrenoceptors) and tracheal chain (β_2-adrenoceptors) were assessed as described by Growcott *et al* (1993). ZD2079 had no effect on basal HR or on the concentration-chronotropic response curve to isoprenaline (concentration ratio=2.7±0.3, n=4). In trachea, ZD2079 (10μM) decreased spontaneous tone by 24.8±6.1% and did not shift the isoprenaline concentration-relaxation response curve (concentration ratio=0.79±0.1, n=4). ZD2079 was also tested in *in vivo* models of β_2-adrenoceptor agonist activity (Holloway *et al*, 1991). In conscious dogs ZD2079, at doses up to 10mg/kg po, did not affect blood K^+ levels. ZD2079 was of low potency (ED_{50}>20mg/kg, iv; n=3) in a cat soleus model of tremor compared to the standard β_2-adrenoceptor agonist, salbutamol (ED_{50}=0.21μg/kg iv; 95% confidence limits: 0.12-0.38; n=3).

In conclusion, ZD2079 is a novel β_3 adrenoceptor agonist and thermogenic agent. It is a full agonist at the β_3-adrenoceptor in white adipocytes relative to isoprenaline and BRL 37344. In contrast, the β_3-adrenoceptor agonist ICI D7114 is a partial agonist in this model (Quayle *et al*, 1993). ZD2079 has limited effects at β_1- or β_2-adrenoceptors *in vitro* and *in vivo*. ZD2079 may have utility in the treatment of NIDDM and obesity.

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214P THE INFLUENCE OF GLUCOSE AND GLIBENCLAMIDE ON VASCULAR β-ADRENOCEPTOR-MEDIATED RESPONSES

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Relaxation of vascular smooth muscle by ß-adrenoceptor agonists occurs mainly via the activation of adenylate cyclase and an increase in intracellular adenosine 3'5'-cyclic monophosphate (cAMP) in the smooth muscle cell. Activation of ß-adrenoceptors can also promote the opening of K^+-channels in airways smooth muscle (Cook *et al*, 1993).
In the present study we evaluated the influence of glucose and glibenclamide, an ATP-sensitive K^+-channel blocker, on ß-adrenoceptor-mediated responses in vascular smooth muscle.

The thoracic aorta of a Wistar rat of approximately 250 gram was carefully excised, cut in segments of approximately 3 mm long and placed in a 5 ml organ bath at 37°C containing a physiological salt solution (PSS), gassed with 95% O_2 and 5% CO_2. The aortic rings were connected to an isometric force transducer and equilibrated for 60 min at a resting tension of 10 mN. The PSS contained 0 or 5.5 M glucose and the aortic rings were incubated for 1 hour with 0, 0.1, 1 and 3 μM glibenclamide. After precontraction with 1 μM phenylephrine cumulative concentration-response curves for isoproterenol, forskolin and dibutyryl-cAMP were constructed.

The pD_2-value for isoproterenol in PSS without glucose (means ± SEM, 7.19 ± 0.07, n=7) was higher than that obtained in PSS with 5.5 M glucose (6.67 ± 0.12, n=6). Glibenclamide only caused a concentration-dependent decrease in maximal relaxation of aortic rings in PSS without glucose (86.3 ± 6.4%, 73.0 ± 5.5%, 55.7 ± 5.8% and 51.1 ± 4.4% for 0, 0.1, 1 and 3 μM glibenclamide, respectively (n=7-8)). Glucose and glibenclamide had no effect on the forskolin induced relaxation. The concentration-response curves for dibutyryl-cAMP with 0.1 and 3 μM glibenclamide were shifted leftward in PSS with 5.5 M glucose (pD_2 of 4.40 ± 0.10 (n=6) and 4.57 ± 0.16 (n=6), respectively) than those obtained in PSS without glucose (3.95 ± 0.13 (n=9) and 4.07 ± 0.14 (n=10), respectively).

In conclusion, isoproterenol needs the presence of glucose for full vasodilation of vascular smooth muscle cells in rat aorta. These results may indicate that under conditions of impaired ATP-metabolism the effective pathway of ß-adrenoceptor stimulation becomes glibenclamide-sensitive. This effect is apparently not cAMP-dependent.

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215P PHOSPHODIESTERASE IV INHIBITORS AND β-ADRENOCEPTOR AGONISTS SUPPRESS LIPOPOLYSACCHARIDE-INDUCED TUMOUR NECROSIS FACTOR-α GENERATION BY HUMAN PERIPHERAL BLOOD MONOCYTES

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Tumour necrosis factor-*a* (TNF*a*) is a 17 kDa polypeptide hormone that is one of the most abundant products secreted by activated macrophages and monocytes (see Beutler & Cerami, 1989). In general, TNF*a* may be considered a pro-inflammatory cytokine that is implicated in a number of diseases including asthma and endotoxic shock. This abstract describes the effect of the β_2-adrenoceptor agonists, salbutamol and procaterol, and a number of isoenzyme-selective phosphodiesterase (PDE) inhibitors, on lipopolysaccharide (LPS)-induced TNF*a* biosynthesis from human peripheral blood monocytes.

Human monocytes were purified from the venous blood of normal individuals and cultured in 24-well plates in FCS-supplemented RPMI 1640. Cells were stimulated with LPS (from *Salmonella enteritidis*) in the absence and presence of the drugs under investigation, and the release of TNF*a* into the culture medium was determined at 18 hrs by a sandwich ELISA (M. Imrie & A. R. Greening, Department of Medicine, Western General Hospital, Edinburgh).

Pre-treatment of monocytes for 20 min with rolipram (PDE IV inhibitor), denbufylline (PDE IV/V inhibitor) and benafentrine (PDE III/IV inhibitor) completely suppressed LPS (3 ng/ml; EC_{84})-induced TNF*a* generation in a concentration-dependent manner. The inhibition produced by rolipram was biphasic and could be resolved into both high potency (EC_{50H} = 226.1 ± 89.3 nM; 79.5 ± 5.7%, n = 3) and low potency (EC_{50L} = 230.6 ± 190 μM; 20.5 ± 5.7%, n = 3) components. In contrast, the concentration-response curves which described the inhibition of TNF*a* generation by denbufylline and benafentrine were monophasic from which EC_{50} values of 341.8 ± 69.8 nM (n = 3) and 5.5 ± 2.4 μM (n = 3) were interpolated respectively. Neither SK&F 95654 (PDE III inhibitor) nor zaprinast (PDE V inhibitor) significantly affected LPS-induced TNF*a* biosynthesis at concentrations upto 100 μM. Pretreatment of monocytes for 5 min with salbutamol and procaterol resulted in a concentration-dependent inhibition of LPS-induced TNF*a* generation with EC_{50} values of 40.5 ± 10.1 nM (n = 3) and 7.7 ± 2.9 nM (n = 3) respectively. Contrary to the results obtained with rolipram, denbufylline and benafentrine, the β-adrenoceptor agonists produced only a partial inhibition of TNF*a* biosynthesis (maximum inhibition: salbutamol 42.0 ± 3.0 %, n = 3; procaterol 37.0 ± 5.5 %, n = 3). Furthermore, a low concentration of rolipram (100 nM) which reduced LPS-induced TNF*a* generation by ~ 20%, failed to potentiate the inhibitory effects of salbutamol (EC_{50} = 17.9 ± 2.4 nM, n = 3) and procaterol (EC_{50} 4.36 ± 1.1 nM, n = 3) on monocyte cytokine production. Indeed, rolipram interacted with both salbutamol and procaterol in a purely additive manner (maximum inhibition: salbutamol: 70.0 ± 2.3 %, n = 3; procaterol 75.1 ± 1.2 %, n = 3).

It is concluded that β-adrenoceptor agonists and inhibitors of the PDE IV isoenzyme family suppress LPS-induced TNF*a* biosynthesis from human peripheral blood monocytes. The inability of rolipram to potentiate the inhibitory effect of both salbutamol and procaterol suggests that β-adrenoceptor agonists may attenuate TNF*a* generation in this cell type by a cyclic AMP-*independent* mechanism.

Supported by the MRC, the British Lung Foundation and the Otsuka Pharmaceutical Company.

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216P 'FUNCTIONAL INHIBITION CURVES' PROVIDE FURTHER EVIDENCE FOR HETEROGENEITY OF α_1-ADRENOCEPTORS IN THE RAT AORTA

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Previously, the analysis of the competitive antagonism of noradrenaline (NA) produced by preincubation with antagonists suggested the existence of a heterogeneous population of α_1-adrenoceptors in the rat aorta (Van der Graaf et al., 1993). Two of the seven antagonists behaved simply, five produced steepening of the agonist concentration-effect (E/[A]) curves and, of these, two gave flat Schild plot slopes. Recently, Lazareno & Birdsall (1993) suggested that accurate estimation of antagonist affinity and Schild plot slope parameters may be obtained by analysis of 'functional inhibition curves' and that this method may be used to enhance conventional Schild analysis for the detection of multiple receptor subtypes. We now report the results obtained using this method with the same seven antagonists. After removal of the endothelium, 4mm ring segments of thoracic aorta from male Wistar rats (225-300g) were mounted in modified Krebs' buffer (2g tension, 37°C, gassed 95%O_2/5% CO_2) with low (0.25mM) [Ca^{2+}] to prevent spontaneous phasic contractions. 30μM cocaine and 6μM timolol (90 min incubation) were present in all experiments. In each tissue, by cumulative dosing, single NA E/[A] curves were obtained up to 3μM, a concentration which produced a sustained near maximal response, followed by antagonist inhibition curves. The E/[A] data in the absence and presence of antagonist were fitted simultaneously to a form of the Schild equation (Lazareno & Birdsall, 1993) to provide estimates of the antagonist affinity and Schild plot slope parameters. With the possible exception of 5-methylurapidil, the slope parameter estimates were significantly greater than unity:

	Schild slope parameter (*P<0.05)	pA_2	n
5-methylurapidil	1.29±0.15	8.07±0.10	4
tamsulosin (YM617)	1.94±0.12*	9.91±0.09	6
prazosin	1.55±0.06*	9.74±0.03	6
WB-4101	1.18±0.05*	9.00±0.05	6
spiperone	2.16±0.15*	8.75±0.07	6
HV723	1.34±0.10*	8.86±0.07	5
phentolamine	1.32±0.09*	8.03±0.07	6

The slope parameters estimated by this method were significantly greater than those obtained previously using conventional Schild analyses (Van der Graaf et al., 1993), but the data could be accounted for by the same 2-receptor model as employed in the aforementioned study. We conclude that this represents further evidence for heterogeneity of α_1-adrenoceptors in the rat aorta and that the 'functional inhibition curve' method is indeed a sensitive indicator of these conditions. However, these data suggest that this method need not yield valid Schild plot slope parameter estimates in tissues with mixed receptor populations.

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217P α_2-ADRENOCEPTOR ACTIVATION INCREASES VOLTAGE-DEPENDENT CALCIUM CHANNEL CURRENTS IN HUMAN ARTERIAL SMOOTH MUSCLE CELLS

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Contractions produced by postjunctional α_2-adrenoceptor activation in human subcutaneous resistance arteries are predominantly dependent on extracellular calcium (Parkinson *et al*, 1991). The type(s) of calcium channels involved in the influx of calcium has not yet been investigated, although voltage-operated calcium channels (VOCs) may be involved as responses to the α_2-adrenoceptor agonist BHT933 (BHT) have been shown to be inhibited by the dihydropyridine calcium antagonist, amlodipine (Garcha *et al*, 1991). To investigate this further, we examined the effect of BHT on calcium channel currents (I_{Ba}) in human arterial cells.

Cells were isolated enzymatically from human omental arteries (n=5). I_{Ba} was measured using whole cell voltage clamp techniques (Hamill *et al*, 1981), with 110mm Ba^{2+} as the charge carrier. Data are means ± s.e.means of n observations.

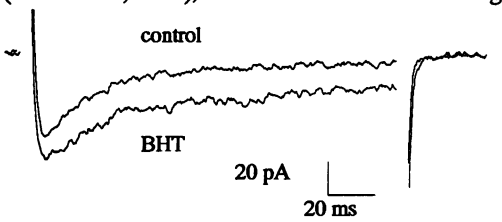


Figure: An example trace showing the enhancement of I_{Ba} induced by α2-adrenoceptor activation. The omental arterial cell was voltage clamped at a membrane potential of -60mV and depolarized to +20mV for 200ms to evoke I_{Ba} (control). Following addition of 10μM BHT the depolarization step was repeated (BHT). A calibration bar is shown below the current traces. The result is representative of 5 similar experiments.

10μM BHT increased peak I_{Ba} evoked by a 200ms depolarization to +30mV from -60mV by 54±19% (n=5), although the shape of the current-voltage relationship was unaffected. Steady-state inactivation of I_{Ba} appeared unaffected by BHT; the membrane potential at which 50% inactivation of I_{Ba} occurred being similar in the presence (-9.2±0.2mV, n=3) or absence (-8.9±1.0, n=3) of BHT.

Increased influx of Ca^{2+} through VOCs may mediate the response to activation of α_2-adrenoceptors on human arterial smooth muscle cells. The mechanism by which α_2-adrenoceptors increase I_{Ba} requires further study.

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218P INFLUENCE OF THE ENDOTHELIUM ON ANGIOTENSIN II-INDUCED CONTRACTIONS IN RABBIT ISOLATED RENAL ARTERY PREPARATIONS

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Several studies have documented enhanced contractile responses in blood vessels to angiotensin II after removal of the endothelium (Gruetter et al., 1988; Bullock et al., 1986), suggesting a potential negative-feedback role for the endothelium in modulating the effects of angiotensin II. The present study was conducted to further evaluate the influence of endothelium on angiotensin II-induced contraction in rabbit isolated renal artery preparations.

Isolated renal artery ring preparations of the rabbit were set up as described previously (Tadipatri et al., 1993) and either endothelium-intact or -denuded ring preparations were suspended in an organ bath with Krebs solution at 37 °C and gassed with 95 % O_2 and 5 % CO_2. Isometric force was measured and recorded. After 1 h of equilibration at 2 g of resting tension, the preparation was challenged with a depolarizing potassium solution as a test for viability and then washed and re-equilibrated with Krebs buffer for 1 h. Cumulative concentration-effect curves for angiotensin II were then established. The preparations were washed again and re-equilibrated for another 1 h. After the tissue had been incubated with vehicle or with different drugs for 30 min the second concentration-response curve for angiotensin II was constructed. In the endothelium-intact renal artery angiotensin II (10^{-9}-10^{-7} M) caused concentration-dependent increases in contractile force by maximally 0.74±0.05 g (n=6). Removal of the endothelium significantly enhanced the angiotensin II-induced maximal contractions (3.91±0.19 g, n=6, $P<0.01$). Indomethacin (10^{-5} M) did not alter angiotensin II-induced contractions in endothelium-intact preparations (0.76±0.06 g, n=7, $P>0.05$). However, methylene blue (10^{-6} M) significantly enhanced angiotensin II-induced contractions (2.10±0.10 g, n=6, $P<0.01$). N^G-methyl-l-arginine (NMA, $5X10^{-5}$ M) significantly enhanced angiotensin II-induced contractions (1.63±0.11 g, n=6, $P<0.01$) in rabbit renal artery with, but not without, endothelium. L-arginine (10^{-3} M), but not D-arginine (10^{-3} M), reversed the NMA-induced enhancement of the angiotensin II-induced contraction (0.98±0.13 g, n=6).

The present findings demonstrate that angiotensin II-induced contractions in rabbit renal artery are strongly subject to the influence of the endothelium. The release of endothelium-derived relaxant factor (EDRF), rather than cyclooxygenase products, appears to be involved in mediating the inhibitory influence of the endothelium. Nitric oxide (NO) derived from endothelium may play a main role in inhibiting angiotensin II-induced contractions in rabbit renal artery, and L-arginine is likely to be the precursor for NO synthesis in the endothelium.

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219P ANGIOTENSIN II GENERATION IN ISOLATED PORCINE CORONARY AND CAROTID ARTERIES

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According to the classical concept, angiotensin I (AI) is converted to angiotensin II (AII) by endothelial-bound angiotensin converting enzyme (ACE). Recent studies indicate that several other enzymes, e.g. human heart chymase (Urata et al, 1993) and cathepsin A (Miller et al, 1988) may also be involved in the generation of AII. Moreover, the endothelium may not be the only site of AI-to-AII conversion (Pipili et al, 1989). It was the aim of the present study to investigate 1) whether ACE is the only AII-generating enzyme in the vessel wall, and 2) whether vascular ACE is limited to the endothelium. We developed an in vitro model that allowed us to study the metabolism of ^{125}I-angiotensin I (^{125}I-AI) in isolated coronary and carotid arteries, obtained from pigs and perfused at 37 °C with oxygenated Krebs-Henseleit buffer at a constant flow rate of 1 ml/min. ^{125}I-AI was added either to the perfusion fluid flowing through the vessel ('lumen') or to the fluid surrounding the mounted perfused vessel ('bath'), in the presence or absence of the ACE-inhibitor captopril. Samples for the measurement of radiolabeled ^{125}I-AI and ^{125}I-AII were taken from both bath and lumen at various times after the addition of ^{125}I-AI. First order rate constants for the metabolism of ^{125}I-AI by conversion into ^{125}I-AII (k_2) or by degradation into metabolites other than ^{125}I-AII (k_1) were calculated from the results.

Table 1. ***First order rate constants for degradation and conversion of ^{125}I-ANG I in isolated vessels (values are mean and SD).***

		Without captopril			With captopril		
		n	k_1 (min^{-1})	k_2 (min^{-1})	n	k_1 (min^{-1})	k_2 (min^{-1})
Coronary artery	Lumen	10	0.0113±0.0167	0.0115±0.0185	3	0.0068±0.0063	0.0006±0.0001
	Bath	8	0.0059±0.0022	0.0039±0.0022	3	0.0031±0.0030	0.0003±0.0002
Carotid artery	Lumen	7	0.0130±0.0162	0.0396±0.0538	3	0.0075±0.0051	0.0013±0.0008
	Bath	6	0.0039±0.0022	0.0033±0.0019	3	0.0028±0.0006	0.0003±0.0001

^{125}I-AI was metabolized fastest when added to the lumen (Table 1). Diffusion of intact ^{125}I-AI or ^{125}I-AII through the vessel wall (from lumen to bath or vice versa) was only marginal (< 5 %). In the lumen 50-60 %, and in the bath 30-40 % of ^{125}I-AI metabolism was due to conversion to ^{125}I-AII; captopril blocked ^{125}I-AII formation in both bath and lumen virtually completely and prolonged the half life of ^{125}I-AI. It is concluded that, in porcine coronary and carotid arteries, 1) ACE is the only enzyme that converts AI to AII, and 2) conversion is not limited to the endothelium but also occurs in the adventitia and/or media.

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220P ENDOTHELIUM-DEPENDENT, NITRIC OXIDE MEDIATED INHIBITION OF ANGIOTENSIN II-INDUCED CONTRACTIONS IN RABBIT AORTA

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The role of the endothelium in angiotensin II (Ang II)-induced contractions in rabbit isolated aorta was investigated. Destruction of the endothelium significantly shifted the concentration-response curve for Ang II to the left in a nonparallel manner and enhanced the maximal response (E_{max}). The EC_{50} and E_{max} values obtained in the aortic rings with functional endothelium were $2.4\pm0.13\times10^{-9}$ mol/l and 45±4.5 mN, respectively. After removal of the endothelium these values amounted to $1.2\pm0.14\times10^{-9}$ mol/l and 57±5.5 mN (n=8, $p<0.05$), respectively. Indomethacin (10^{-5} mol/l), a cyclo-oxygenase inhibitor, did not significantly alter the concentration-dependent response to Ang II in the presence of endothelium.
Three inhibitors of nitric oxide synthase (N^G-monomethyl-L-arginine, L-NMMA; N^G-nitro-L-arginine, L-NNA and N^G-nitro-L-arginine methyl ester, L-NAME, at 10^{-4} mol/l) (Rees et al., 1990) alone induced small but significant endothelium-dependent rises in basal tension, suggesting the occurrence of basal release of nitric oxide (NO) in rabbit aortic rings. All of these three compounds caused a similar endothelium-dependent potentiation of Ang II-induced contraction in the aortic rings with, but not in those without endothelium. The E_{max} values obtained in the presence of L-NMMA, L-NNA and L-NAME were 123±2.6 %, 124±4.9 % and 128±3.6 % ($p<0.05$, n=5-6) of the control maximal value, respectively. These effects were reversed by L-arginine (3×10^{-3} mol/l) but not by D-arginine (3×10^{-3} mol/l). These data suggest that locally produced endothelium-derived relaxing factor (EDRF/NO) from L-arginine in endothelium cells of the rabbit aorta attenuates the contraction induced by Ang II. Under our experimental conditions, the inhibition of ACh-induced relaxation caused by L-NMMA was almost completely reversed by L-arginine, whereas the inhibitory effects of L-NNA and L-NAME on the relaxation induced by ACh were only partially antagonised by the simultaneous treatment with L-arginine (3×10^{-3} mol/l). Ang II at a concentration range of 10^{-16} to 10^{-6} mol/l did not relax the endothelium-intact aortic rings precontracted with phenylephrine (2×10^{-7} mol/l). In the presence of endothelium, the Ang II subtype 2 receptor antagonist PD 123177 caused neither relaxation of the rings precontracted with phenylephrine nor an alteration of the concentration-response curve for Ang II, indicating that AT_2 receptors are not involved.

In conclusion, these results provide evidence for the occurrence of an endothelium-dependent inhibition of Ang II-induced contractions in the rabbit aorta. They support the hypothesis that basal or stimulated release of NO attenuates the contraction induced by Ang II in this preparation.

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221P INHIBITION OF NITRIC OXIDE FORMATION WITH AMINOGUANIDINE ATTENUATES THE DELAYED CIRCULATORY FAILURE IN ENDOTOXIC SHOCK IN THE RAT

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An enhanced formation of nitric oxide (NO) by the constitutive NO synthase (cNOS) and the inducible one (iNOS) importantly contributes to the early (cNOS) and delayed (iNOS) circulatory failure in endotoxic shock (Szabo et al., 1993). Here we investigate the effects of aminoguanidine (AG), a selective inhibitor of iNOS (Corbett et al., 1992; Misko et al., 1993), on the circulatory failure and the expression of iNOS caused by endotoxin in the anaesthetized rat.

Male Wistar rats (240-300 g) were anaesthetized with sodium thiopental (120 mg kg^{-1} i.p.) and instrumented for the mesurement of mean arterial blood pressure (MAP) and heart rate (HR). Lipopolysaccharide (endotoxin, LPS, 10 mg kg^{-1} i.v.) was administered in vehicle-treated rats (control, n=10) and in rats pretreated with AG (15 mg kg^{-1} i.v. at 20 min prior to LPS, n=10). The dose did not significantly affect MAP. Pressor responses to noradrenaline (NA, 1 μg kg^{-1} i.v.) were assessed prior to, and 60, 120 and 180 min after LPS injection. Thoracic aortae and lungs were obtained at 180 min after injection of LPS from vehicle- and AG-treated rats. Isometric tension of aortic rings was measured and dose-response curves to noradrenaline (NA, 10^{-9} to 10^{-6} M) were obtained. Calcium-dependent (cNOS) and calcium-independent (iNOS) NOS activity was measured in lung homogenates by the conversion of ^{3}H-L-arginine to ^{3}H-L-citrulline (Szabo et al., 1993).

LPS caused a fall in MAP from 115±5 (control) to 74±8, 78±6, 87±6 and 79±9 mmHg at 5, 60, 120 and 180 min, respectively ($p<0.05$; n=10). Pressor responses to NA were significantly reduced from 30±2 mmHg (control) to 11±1, 16±2 and 18±4 mmHg at 60, 120 and 180 min after LPS, respectively ($p<0.05$; n=10). Thoracic aortae obtained from rats at 180 min after LPS showed a significant reduction in the contractile responses elicited by NA. Endotoxaemia for 3 h resulted in a significant increase in iNOS activity in the lung from 0.6±0.2 pmol mg^{-1} min^{-1} (control, n=4) to 4.8±0.3 pmol mg^{-1} min^{-1} ($p<0.05$, n=6). Animals pretreated with AG maintained higher MAP at 60 (90±4 mmHg), 120 (106±3 mmHg) and 180 min (103±3 mmHg) after LPS (n=10, $p<0.05$ when compared to LPS-control at the same time point). Similarly, the pressor responses elicited by NA in AG-pretreated LPS-rats were significantly greater at 60 (18±1 mmHg), 120 (23±2 mmHg) and 180 min (31±4 mmHg) than in vehicle-treated LPS-rats (n=10, $p<0.05$). Pretreatment with AG also significantly attenuated the hyporeactivity of aortic rings to NA measured *ex vivo*. Moreover, AG attenuated the increase in iNOS activity caused by endotoxaemia in the lung by 44±5% (n=6, $p<0.05$). When added *in vitro* to lung homogenates prepared from LPS-rats, AG (10^{-8} to 10^{-3} M) caused a dose-related inhibition of iNOS activity (n=3-6).

Thus, AG inhibits iNOS activity and attenuates the delayed circulatory failure (hypotension and vascular hyporeactivity) caused by LPS in the rat suggesting that AG may be useful in the therapy of septic shock.

This work was supported by a grant from Glaxo Group Research Ltd. C.C.Wu is supported by the NDMC of Taiwan, ROC. C.Szabó is a fellow of Lloyd's of London Tercentenary Foundation.

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222P 9-HYDROXY- AND HYDROPEROXYLINOLEIC ACID INHIBIT ENDOTHELIUM-DEPENDENT RELAXATION IN RABBIT ISOLATED AORTA

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During the development of atherosclerosis endothelium-dependent vasodilation mediated by nitric oxide is impaired (Flavahan,1992). It has been suggested that this dysfunction may result from the accumulation of oxidatively-modified low-density lipoproteins (LDL) in the arterial wall (Jacobs *et al.* 1990), a process known to contribute to the pathogenesis of atherosclerosis. The exact mechanism by which LDL becomes oxidised remains unclear but involves lipid peroxidation of linoleic and archidonic acids producing their hydroperoxy and hydroxy fatty acids. Previous studies have shown that Cu^{2+}- oxidised LDL inhibit ACh - evoked relaxations in isolated rabbit aorta (Jacobs *et al.* 1990) and coronary arteries (Buckley *et al.* 1993). The aim of this study was to examine the vascular effects of the hydroxy (HODE) and hydroperoxy derivatives (HpPODE) of linoleic acid present in oxidised LDL to see whether they might contribute to the inhibition of relaxation.

Endothelium-dependent relaxation was assessed in intact aortic rings from New Zealand White rabbits as previously described (Jacobs *et al.* 1990). Tissues were precontracted with phenylephrine (0.1-0.3 μ M) and relaxed with cumulative concentrations (1nM-10 μM) of acetylcholine (ACh). After a 30 min washout period, tissues were exposed to 9(S)-HODE, 9(S)-HpODE or 13(S)-HODE and the contraction/relaxation cycle was repeated. A third cycle of contraction relaxation-was carried out after a further washout to determine the reversibility of any effects.

In the presence of 9-HODE (5μM), ACh evoked relaxations were attenuated. This was demonstrated by a significant reduction in the maximal relaxation to ACh from 91.6 ± 1.6 % in control tissues to 80.0 ± 2.4 % in rings treated with 9-HODE ($P<0.05$; n=7). After washout, responses were restored to control values (92 ±1.9%). Addition of 9-HpODE or 13-HODE produced similar degrees of inhibition.: 11.8 ± 1.4 % (n=4) and 14.2 ± 1.7 % (n=5) respectively. Preincubation of the tissues with indomethacin (10 μM) for 15 min did not alter the degree of inhibition caused by the presence of 9-HODE as shown by the maximal relaxations of the control (94.5 ± 2.3 %) and treated rings (80.5 ± 4.7 %; n=4,$P<0.05$). Preincubation of of the tissues with 1 μM chelerythrine chloride, a protein kinase C inhibitor (Herbert *et al.* 1990) prevented the inhibition of relaxations by 9-HODE: 92.3 ± 1.9 % and 93.8 ± 2.4 % for treated and control tissues respectively. None of the hydroxy and hydroperoxy acids studied had any effect on endothelium-independent relaxations evoked by glyceryl trinitrate in tissues either with or without endothelium.

In conclusion, this study demonstrates that 9-HODE, 9-HpODE and 13-HODE inhibit endothelium-dependent relaxations mediated by nitric oxide by a pathway dependent on the activation of protein kinase C. Products of the cyclooxygenase pathway do not play a role in the inhibition. The continual generation of these fatty acids in the vessel wall may contribute with other constituents of oxidised LDL to the impairment of nitric oxide mediated endothelium-dependent vasodilation.

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223P MODULATION OF NEUROGENIC OEDEMA FORMATION BY NITRIC OXIDE SYNTHASE INHIBITORS IN THE RAT

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The nitric oxide (NO) synthase inhibitor, L-N^G-nitro arginine methyl ester (L-NAME) inhibits capsaicin-induced neuropeptide-dependent increase in blood flow in rabbit skin (Hughes & Brain, in press). We investigated the possibility that NO can modulate neurogenic oedema formation in the rat. The effect of L-NAME and the alternative NO synthase inhibitor, 7-nitro indazole (7-NI, Moore *et al.*, 1993), on oedema formation in response to electrical stimulation (10V, 2Hz, 1ms for 5min) of the saphenous nerve was quantified by the extravascular accumulation of intravenously injected ^{125}I-albumin (Escott & Brain, 1993).

Table 1: Effect of L-NAME and 7-NI on neurogenic oedema formation. Results are a ratio of oedema (stimulated/sham paw), mean±s.e.mean.

Control (i.v.)		n	Test agents (i.v.)		n	p (t-test)
Saline (0.8ml/kg)	6.16±1.74	5	L-NAME (10mg/kg)	7.38±1.77	7	0.65
Saline (0.8ml/kg)	8.03±1.04	6	L-NAME (100mg/kg)	2.83±0.30	6	0.02
Saline (0.8ml/kg)	7.19±1.24	4	D-NAME (100mg/kg)	8.00±1.67	4	0.74
Control (i.v.)		n	Test agents (i.v.)		n	p (t-test)
Na_2CO_3 (5ml/kg)	6.28±1.07	7	7-NI(10mg/kg)	2.57±0.51	8	0.02
Na_2CO_3 (5ml/kg)	7.49±1.71	5	7-NI(3mg/kg)	4.75±0.67	6	0.15
Na_2CO_3 (5ml/kg)	6.28±1.07	7	6-AI (10mg/kg)	7.02±0.72	6	0.42

L-NAME (100mg/kg) and 7-NI (10mg/kg) significantly inhibited saphenous nerve-induced oedema formation. However, D-NAME (100mg/kg) and 6-amino indazole (6-AI, 10mg/kg), which exhibits minimal NO synthase inhibitory activity in vitro (Moore *et al.*, 1993), did not significantly influence oedema formation.

In further experiments, L-NAME (100mg/kg, i.v.) but not 7-NI (10mg/kg, i.v.) significantly increased systemic blood pressure (MAP, before 115±18 and 5 min after L-NAME 174±18 mmHg n=6, $p<0.05$; before 96±9 and 5 min after 7-NI 102±10 mmHg, n=6, $p>0.05$). Concomitant paw skin blood flow measurements (by laser Doppler flowmetry; measured as arbitary flux units) revealed that neither L-NAME nor 7-NI significantly ($p>0.05$) affected basal (before 37±5 and after L-NAME 33±5 flux, n=6; before 31±4 and after 7-NI 29±2 flux, n=6) or CGRP (10pmol/site)-induced (before 95±12 and after L-NAME 128±23 flux, n=6; before 85±10 and after 7-NI 94±13 flux, n=6) cutaneous blood flow.

These results suggest that NO is involved in neurogenic oedema formation but it is unlikely that L-NAME and 7-NI act as a consequence of changes in blood flow. These results are compatible with the possibility that NO plays a role in the release of neuropeptides from sensory nerves.

RK is supported by a Pfizer Ph.D studentship.

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224P INDUCTION OF NITRIC OXIDE SYNTHASE IN CULTURED VASCULAR SMOOTH MUSCLE CELLS: THE ROLE OF PROTEIN KINASES

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Recent work has suggested a major role for nitric oxide (NO) in the pathogenesis of septic shock. One isoform of nitric oxide synthase (iNOS) is particularly interesting in this regard. Its expression in the vessel wall is inducible by endotoxin and pro-inflammatory cytokines resulting in potent vasodilatation which is refractory to vasopressor drugs (Beasley, 1989). Although the intracellular signalling involved in this induction of iNOS is poorly understood iNOS contains several phosphorylation sites (Lowenstein et al., 1992). The purpose of this study was to investigate the effect on iNOS induction in cultured vascular smooth muscle cells (VSMC) of a variety of agents with known effects on protein kinase function.

Cultured rat aortic smooth muscle cells that were passage 4-10 were used throughout. NOS activity was assessed by measurement of nitrite (NO_2^-) accumulation in the culture medium using the Griess reaction. iNOS mRNA was studied in VSMC by reverse transcription-polymerase chain reaction amplification (PCR).

Exposure of cultured VSMC to recombinant human IL-1β (1-100ng/ml) resulted in a substantial increase in NO_2^- accumulation; with 10ng/ml IL-1β NO_2^- accumulation was (mean±s.e.mean) 2.42±0.26 nmol/10^5 cells/24h vs. 0.29±0.022 in control wells (n=28). Dibutyryl cyclic AMP (dBcAMP) enhanced IL-1β-induced (10ng/ml) NO_2^- accumulation in a concentration-dependent manner (enhancement over control with dBcAMP 0.01, 0.1 and 1 mM was 131±15, 255±26 and 393±71%, n=6-8). Dibutyryl cyclic GMP (1mM) had no effect. Similarly, both forskolin (1-10 μM) and a phosphodiesterase inhibitor, Ro 20-1724 (1-100 μM), enhanced IL-1β-induced NO_2^- accumulation in a concentration-dependent manner (10μM forskolin 259±48%, n=5; 10μM Ro 20-1724 170±25%, n=4). Isoprenaline also enhanced NO_2^- production by IL-1β-stimulated VSMC dose-dependently (0.1-10 μM), while an α-adrenergic agonist phenylephrine (0.1-10 μM) had no effect. Activation of protein kinase C (PKC) with phorbol 12-myristate 13-acetate (PMA) significantly inhibited NO_2^- accumulation (with PMA 1, 10 and 100nM accumulation was 72±12, 52±15 and 41±6.3% of control, n=4) while the PKC inhibitor, staurosporine substantially elevated it (10 nM 261±52%, n=7). The phosphatase inhibitor okadaic acid also enhanced the accumulation of NO_2^- (100nM 400±259%, n=3). None of the agents tested had any effect on accumulation of NO_2^- in the absence of IL-1β. In unstimulated VSMC, iNOS transcript was not detectable after 36 cycles of PCR. However, after IL-1β (10 ng/ml) iNOS gene expression was induced time-dependently. Addition of dBcAMP (1 mM) enhanced the level of iNOS mRNA induced by a 4 h exposure to IL-1β some 18-fold (18.3±6.8, n=4).

In summary, induction of NOS activity in VSMC by IL-1β is modulated by protein kinases. The increase in iNOS mRNA levels suggests that at least a part of the effect of dBcAMP may occur at the level of iNOS transcription.

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225P INDUCTION OF N^G-MONOMETHYL-L-ARGININE (L-NMMA) TRANSPORT IN CULTURED ENDOTHELIAL CELLS AND MACROPHAGES

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Synthesis of nitric oxide (NO) from L-arginine is involved in regulating many aspects of cellular function (Moncada et al., 1991). In endothelial cells and macrophages N^Gmonomethyl-L-arginine (L-NMMA) is a potent and selective inhibitor of NO production (Rees et al., 1990), however little is known about the mechanisms regulating its uptake into these cells. Experiments were performed using the human endothelial cell line (SGHEC-7; Fickling et al., 1992) and a murine macrophage cell line (J774 cells). Nitrite production, determined using the Greiss reagent and uptake of L-[^{14}C]NMMA were measured as described previously (Bogle et al., 1992). In endothelial cells uptake of L-NMMA (10 μM) was rapid, linear for 60s and mediated via a saturable transport system with an apparent K_t of 77±2 μM and V_{max} of 1059±72 pmol/mg protein/30s (n=3). Stimulation of endothelial cells with endotoxin (1μg/ml, 6h) significantly increased L-NMMA uptake from 565±34 to 757±49 pmol/mg protein/30s ($P<0.05$, unpaired t-test, n=3). Co-incubation of cells with cycloheximide (1μM) but not dexamethasone (1μM) abolished the endotoxin-induced stimulation of L-NMMA uptake (n=3). In J774 cells, L-NMMA uptake (50μM) was time-dependent and linear for 120s. Stimulation with endotoxin (1μg/ml, 0-8h) resulted in an induction of NO synthase as measured by increased nitrite production and was also associated with induction of L-NMMA uptake (Figure 1). These results demonstrate that L-NMMA uptake is stimulated by endotoxin in both endothelial cells and macrophages. In endothelial cells stimulation of L-NMMA uptake is dependent on protein synthesis but is not sensitive to glucocorticoids. Induced uptake of L-NMMA in cells exposed to endotoxin might provide a mechanism for apparent cell selective inhibition of NO synthase in cells expressing inducible NO synthase.

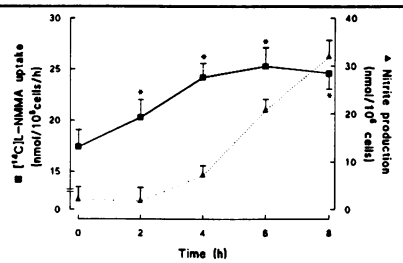


Figure 1 Endotoxin induces NO synthase and also stimulates L-NMMA uptake in cultured J774 cells. Results are mean ± S.E., n=3, * $P < 0.05$, unpaired t-test.

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226P AN INVERSE RELATIONSHIP EXISTS BETWEEN THE SIZE OF RAT PULMONARY ARTERIES AND THEIR RESPONSIVENESS TO CARBACHOL

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There is increasing evidence to suggest a fundamental role for the endothelium-derived relaxing factor, nitric oxide (NO), in maintaining the normally low pulmonary vascular tone (Persson et al., 1990; Yamaguchi et al., 1989). However, it is not known whether the extent to which NO influences tone is a function of vessel size. This study sought to determine the response of pulmonary arteries of different sizes to an endothelium-dependent vasorelaxant, carbachol (CARB) and an endothelium-independent vasorelaxant, sodium nitroprusside (SNP).

Male Wistar rats were killed by stunning and exanguination. Rings from the main pulmonary artery (MPA), 1st and 2nd branches, were isolated, mounted in organ baths under 1 g tension in Krebs' buffer at 37°C, gassed with 95% O_2/5% CO_2. Cumulative concentration-response curves were obtained in the presence of the EC_{75} for phenylephrine (PE) (2.2 x 10^{-7} M), to CARB (10^{-9}-10^{-4} M) and SNP (10^{-11}-10^{-4} M). Relaxant effects were expressed as a % relaxation of PE-induced tone.

An inverse correlation was found between the vessel size and CARB-induced relaxations. Thus, 2nd branch rings displayed significantly greater relaxations in response to CARB than the larger arteries. The maximum relaxations in MPA, 1st and 2nd branches, were 39.5 ± 4.0%, 81.6 ± 2.4% and 102.1 ± 4.5% (mean ± s.e. mean, n=6) ($P<0.001$) respectively. This result is in agreement with a previous study on bovine pulmonary arteries which showed higher cGMP levels in smaller than in larger branches (Ignarro et al., 1987). On the other hand, SNP produced a similar degree of maximum relaxation (≈100%) in the three types of arteries. However, the 2nd branch rings were less sensitive than the larger arteries to the submaximal concentrations of SNP.

The higher sensitivity of the smaller arteries to CARB-induced relaxations may be due to the generation of greater amounts of NO by the endothelium, or to a higher concentration of NO in smooth muscle cells as a result of a thinner vessel wall. The results obtained with SNP exclude the possibility of a higher guanylate cyclase sensitivity in the smaller arteries.

In conclusion, these results suggest that the importance of NO in regulating pulmonary vascular tone is greater in small arteries than in large conduit arteries.

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227P L-NAME ENHANCES VASORELAXATION TO SODIUM NITROPRUSSIDE IN RAT PULMONARY ARTERY RINGS PRECONTRACTED WITH KCl

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Endothelium-dependent (carbachol, CARB) and independent (sodium nitroprusside, SNP) vasorelaxants, which operate through elevating intracellular cGMP levels, are more powerful inhibitors of vascular smooth muscle tone in vessels precontracted with agonists acting through the PI-mechanism (e.g. phenylephrine, PE) than in KCl-precontracted vessels (Lincoln, 1983). The mechanisms underlying this difference are still poorly understood. One reason might be the ability of cGMP to antagonise several of the mechanisms mediating agonist-induced contractions, whereas the voltage-operated channels through which KCl-stimulated Ca^{2+} influx occurs, are insensitive to cGMP (Godfraind, 1986). The bioassay study of Rubanyi (1986) suggesting that KCl stimulates nitric oxide (NO) release, prompted us to investigate the effect of inhibiting NO synthesis with N-nitro-L-arginine methyl ester (L-NAME) on SNP-induced relaxations in pulmonary arterial rings precontracted to a similar extent with PE or KCl. The effect of CARB was also investigated.

Male Wistar rats were killed by stunning and exanguination. Rings from the 1st order branches were isolated, mounted in organ baths under 1 g tension in Krebs' buffer at 37°C, gassed with 95% O_2/5% CO_2. Cumulative concentration-response curves were obtained, in the presence of the EC_{75} for PE (2.2×10^{-7} M) or KCL (27.5 mM) to CARB (10^{-9}-10^{-4} M) and SNP (10^{-11}-10^{-4} M) in the absence and presence of L-NAME (2×10^{-4} M) which was equilibrated with tissues for 10 min before adding drugs. Relaxant effects were expressed as a % relaxation of PE or KCl-induced tone.

The maximum relaxation produced by CARB in PE-precontracted rings was 81.6 ± 2.4% but only 25.6 ± 2.1% (mean ± s.e. mean, n=6) ($P<0.001$) in KCl-precontracted rings. SNP produced a maximum relaxation of 100% (n=6) in PE-precontracted rings which was unaltered by pretreatment with L-NAME. In KCl-precontracted rings, the maximum relaxation induced by SNP was 65.7 ± 5.4% (n=6, $P<0.001$). Inhibition of NO synthesis with L-NAME increased this response to 95.6 ± 5.9% (n=6, $0.01>P>0.001$). These results confirm previous reports in showing a greater relaxant effect of CARB and SNP in PE-precontracted than in KCl-precontracted vessels, but they imply that the limited capacity of CARB and SNP to relax KCl-induced contractions reflects the ability of KCl to stimulate NO release and, therefore, reducing the capacity of the endothelium to generate more NO in response to CARB and decreasing the amount of unstimulated guanylate cyclase available for NO liberated from SNP.

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228P PARTICIPATION OF NITRIC OXIDE IN THE RELAXATION OF THE RAT GASTRIC CORPUS

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Nitric oxide (NO) has been shown to mediate relaxation not only of vascular but also of gastrointestinal smooth muscle. The present study investigated the role of NO in the circular muscle of the rat gastric corpus *in vitro*.

Circular, mucosa-free, strips of rat gastric corpus were suspended in Krebs solution gassed with 95% O_2/5% CO_2 at 37°C for isometric tension recording. In order to block adrenergic mechanisms 5 μM guanethidine was routinely added to the Krebs solution.

Muscle strips, which had been precontracted with 30 μM bethanechol, relaxed in response to electrical field stimulation (EFS; 0.3 ms, 10 Hz and supramaximal voltage for 30 s) to 48 ± 4% (mean ± s.e.mean; n=26) of the previous contraction. This relaxation was concentration-dependently inhibited by the NO-synthase inhibitors L-N^G-nitro-arginine (L-NNA) or L-N^G-nitro-arginine-methyl-ester (L-NAME) in a concentration range of 1 - 300 μM. The D-enantiomers were markedly less active in inhibiting EFS-induced relaxations. The inhibitory effect of 10 μM L-NAME could be prevented by pretreating the strips with 300 μM L-arginine. L-NNA and L-NAME, however, did not influence baseline tension or the bethanechol-induced contraction. Vasoactive intestinal polypeptide (VIP) or sodium nitroprusside dose-dependently relaxed muscle strips precontracted with bethanechol. These relaxations were not affected by 30 μM L-NAME.

In another set of experiments, EFS was delivered without precontracting the strips. In these instances most strips showed a biphasic response consisting of a contraction followed by relaxation. L-NNA or L-NAME (30 μM each) completely blocked the relaxatory and enhanced the contractile component. Furthermore, some strips contracted forcefully at the end of the stimulus. This "off-contraction" was blunted by 30 μM L-NAME. All these effects of the NO-synthase inhibitors were abolished in the presence of 1 μM atropine.

Release of acetylcholine from circular strips of rat gastric corpus was measured as described by Kusunoki *et al.* (1985). The strips were preloaded with [^{3}H]choline, and release of [^{3}H]acetylcholine was evoked by EFS (stimulation parameters as above). Addition of 30 μM L-NAME to the superfusate changed neither the spontaneous nor the electrically evoked release of [^{3}H]acetylcholine.

The results demonstrate a role of NO in the relaxation of the circular muscle of the rat gastric corpus. The relaxation induced by VIP does not involve NO. An interaction of NO with cholinergic mechanisms seems to occur, which, however, does not consist in NO influencing acetylcholine release.

Supported by the Austrian Research Foundation, grant no. P7858, and the Franz Lanyar Foundation.

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229P DISCREPANCY BETWEEN VASORELAXATIONS INDUCED BY N^G-HYDROXY-L-ARGININE AND ITS EFFECTS ON THE FORMATION OF CYCLIC GMP

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The first enzymic step in the production of nitric oxide (NO) from L-arginine results in the formation of N^G-hydroxy-L-arginine (L-HOArg; Stuehr et al., 1991) which is then metabolised to L-citrulline and NO either by NO synthase (NOS) or other enzymes, e.g. cytochromes p450 (Boucher et al., 1992). Interestingly, L-HOArg is a potent endothelium-dependent vasorelaxant of blood vessels (Wallace et al., 1991), whereas L-arginine is not (Palmer et al., 1988; Mitchell et al., 1991), suggesting L-HOArg may have additional actions, or be metabolised by alternative pathways. Indeed, L-HOArg may produce relaxation via three different mechanisms. Firstly, it is a substrate for constitutive NOS present in cultured endothelial cells (ECs). Secondly, it reacts with NO generated by this enzyme to form a potent and more stable vasodilator and thirdly, it releases from EC a relaxing factor, the formation of which is not blocked by inhibitors of NOS (Zembowicz et al., 1991), such as N^G-nitro-L-arginine methyl ester (L-NAME).

Here, we have examined the ability of L-HOArg both to release and to potentiate the effects of NO from bovine aortic ECs grown in culture, using as an assay the accumulation of cyclic GMP (cGMP) in rat foetal lung fibroblasts (RFL-6 cells). In these experiments, Locke's buffer incubated with ECs for 3 min in the absence or presence of agents was transferred onto RFL-6 cells. After a further 3 min the RFL-6 cells were lysed and the intracellular contents of cGMP determined by radioimmunoassay (Ishii et al., 1991). To examine the possibility that L-HOArg scavenges O_2^-, we monitored spectrophotometrically the reduction of ferricytochrome C by O_2^- (generated by pyrogallol) at 550 nm in the presence or absence of L-HOArg or superoxide dismutase (SOD), as a control.

The formation of cGMP in response to basally released NO was increased above basal levels by L-HOArg (10 μM) by 90±32%, SOD (10 U/ml) by 103±46%, and L-HOArg+SOD by 180±58% (n=7-9). Similarly, in the presence of ADP (10 μM), there was a significant increase in the formation of cGMP (ADP, 97±42%; ADP+SOD, 281±61%; ADP+L-HOArg+SOD, 410±37%; n=7-9). The elevations in cGMP accumulation evoked by ADP (30 μM; n=3) or L-HOArg (30 μM; n=3) were inhibited in a concentration-dependent manner by L-NAME (1-100 μM) with approximate IC50s of 3 μM and 10 μM, respectively (n=3 for each). L-NAME (100 μM) or haemoglobin (10 μM) completely blocked elevations in cGMP induced by either ADP or L-HOArg. The reduction of ferricytochrome C by O_2^- was unaffected by L-HOArg but significantly decreased by SOD (n=9-14).

Thus, we confirm our previous observations using the cascade that L-HOArg may both augment the release of NO, and enhance the detection of NO released by endothelial cell agonists. This effect, unlike that of SOD, may not be attributed to scavenging of O_2^-. We also found that L-NAME completely blocked elevations in cGMP caused by L-HOArg, although we have previously shown by cascade bioassay that endothelial-dependent relaxations induced by L-HOArg are not L-NAME-sensitive. This discrepancy suggests that the relaxant effects revealed on the cascade are not entirely cGMP-mediated. Alternatively, it may reflect differences in the activity of ECs under conditions of flow versus stasis.

This work was supported by a grant from Glaxo Research Group Ltd. T.S. is a recipient of a British Heart Foundation grant.

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230P CYTOTOXIC ACTIONS OF NITRIC OXIDE DONORS ON RAT ISOLATED INTESTINAL EPITHELIAL CELLS: EFFECTS OF SUPEROXIDE DISMUTASE AND INDOMETHACIN

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Induction of nitric oxide (NO) synthase in the rat small intestine by endotoxin (Salter et al, 1991) is associated with both microvascular injury *in vivo* (Boughton-Smith et al, 1993) and reduced viability of epithelial cells *in vitro* (Tepperman et al, 1993), effects attributed to the cytotoxicity of high concentrations of NO or its reactive products. The actions of exogenous NO on the viability of intestinal epithelial cells *in vitro* have now been evaluated by the use of NO donors.

Epithelial cells were isolated from rat proximal small intestine by luminal incubation with EDTA and dithiothreitol, then washed and cell viability subsequently determined by trypan blue exclusion (Tepperman et al, 1993). Incubation with the NO donors nitroprusside, the nitrosothiols S-nitroso-N-acetyl penicillamine (SNAP) or S-nitroso-N-glutathione (SNOG) at 37°C (100-1000 μM for 60 min) caused a concentration-dependent, significant ($P<0.01$ by ANOVA) increase in the number of non-viable cells, from the control value of 9±2% to 50±1%, 49±4% and 49±5% of total respectively (mean±s.e.mean, n=5-10) at a concentrations of 500 μM of each agent. In control studies, incubation with potassium ferricynanide, SNAP depleted of NO (by prior incubation at 37°C for 48h) or glutathione at such concentrations had no significant action on cell viability. Concurrent incubation with superoxide dismutase (SOD; 100 unit ml^{-1}), which itself did not affect viability, significantly enhanced the cytotoxic actions of these NO donors, the number of non-viable cells with SNOG (250 μM) being thus elevated from 35±1 to 63±3% of total ($P<0.001$). In addition, incubation with the cyclo-oxygenase inhibitor indomethacin (10 μM), which itself elevated the number of non-viable cells under resting conditions (to 22±2%, $P<0.01$), further increased the injurious action of these NO donors, the number of non-viable cells with SNAP (250 μM) being thus increased from 30±4% to 44±2% of total ($P<0.01$).

These findings indicate that these NO donors can exert damaging actions *in vitro* on intestinal epithelial cells, an effect that is modulated by endogenous protective prostaglandins. The observation that SOD augmented such injury suggests that the cytotoxic actions of the liberated NO are attenuated by the superoxide anion. Such actions however, obscure interpretation of the role of the peroxynitrite moiety and its reactive breakdown products, formed from NO and superoxide (Beckman et al, 1990), in such epithelial damage.

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231P PROLONGED INHIBITORY EFFECT OF L-N^G-NITRO ARGININE METHYL ESTER (L-NAME) ON MEDIATOR-INDUCED EXTRAVASATION IN GUINEA-PIG SKIN

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Co-injection of the nitric oxide synthase inhibitor L-NAME inhibits bradykinin (BK) induced plasma protein extravasation (PPE) in guinea pig skin (Paul et al., 1992; Teixeira et al., 1993). In this study, we have assessed the duration of the inhibitory effect of L-NAME in comparison with that of the α-adrenoceptor agonist phenylephrine (Phe) in guinea pig skin. Intradermal (i.d.) injections (0.1ml) were made in duplicate in shaved flank skin according to a balanced Latin square design. PPE was measured as local accumulation of intravenously injected ^{125}I-albumin over a 40min period, calculated as μl plasma (^{125}I counts in skin / ^{125}I counts in 1μl plasma) and corrected by subtracting leakage in diluent (phosphate buffered saline; PBS) or drug control sites. Mean ± s.e.mean values are shown. The significance of differences between means was assessed by analysis of variance followed by Tukey's test. n = number of animals.

Co-injection of L-NAME (0.1μmol site^{-1}) with BK (0.75nmol site^{-1}) was compared with pre-injection of L-NAME from 0.5 to 16h prior to BK (each, n=6). In every case, BK and (BK+ L-NAME) sites were pre-injected with PBS at the appropriate time and ^{125}I-albumin was injected 0.5h prior to the final i.d. injections. In sites injected with L-NAME 0.5h earlier, BK induced PPE was 26.0 ± 6.2μl which was significantly ($p<0.01$) less than in PBS pre-treated sites (115.7 ± 17.6μl) and also significantly ($p<0.05$) less than in (BK+L-NAME) sites (57.7 ± 9.1μl). Sites pre-injected with L-NAME (8h) still showed significantly ($p<0.05$) reduced PPE (62.9 ± 5.2μl) compared with controls (92.1 ± 8.2μl) but were now significantly ($p<0.01$) greater than (BK+L-NAME) sites (37.4 ± 3.3μl). The inhibitory effect of L-NAME was not significant at 16h. Similar results were obtained with histamine induced PPE. In contrast, PPE in sites pre-injected with Phe (5nmol site^{-1}) 0.5h prior to BK was not significantly reduced compared with controls though (Phe+BK) sites were significantly ($p<0.01$) inhibited (BK controls : 100.4 ± 10.2μl; Phe pre-injection : 83.9 ± 5.6μl; Phe+BK : 49.6 ± 7μl; n=5).These observations show that the inhibitory effect of L-NAME persists for > 8h following i.d. injection in the guinea pig and that this prolonged effect is not seen with the vasoconstrictor phenylephrine.

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232P L-ARGININE AND PLASMA EXTRAVASATION IN RABBIT SKIN: AN EFFECT ON RESPONSES TO BRADYKININ BUT NOT ON THOSE TO ANTIGEN OR HISTAMINE

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In guinea pig and rabbit skin, local injection of the nitric oxide synthase inhibitor L-N^G-nitro arginine methyl ester (L-NAME) inhibited plasma protein extravasation (PPE) induced by a variety of stimuli (Paul et al., 1992a). In guinea pigs, L-arginine (arg) enhanced PPE induced by bradykinin (BK) but inhibited histamine (HA) induced PPE (Paul et al., 1992b). A possible antihistaminic effect of arg appeared to be confirmed by studies with guinea pig ileum (Khawaja et al., 1993). In the present study, we have examined the effect of L-arg on PPE responses in rabbit skin and have included some further data in guinea pig skin for comparison.

Intradermal injections (0.1ml) were made in balanced blocks in the shaved flank skin of male Dunkin Hartley guinea pigs and NZW rabbits. PPE was measured as local accumulation of intravenous ^{125}I-albumin. Mean ± s.e. mean values (μl plasma) were corrected by subtracting the appropriate controls. The significance of differences between means was assessed by analysis of variance followed by Tukey's test. n = number of animals. In the skin of rabbits immunised to produce predominantly IgE antibodies (n=4), injection of antigen (Alternaria tenuis, 400 and 133 PNU site^{-1}) produced PPE responses of 115 ± 18 and 57 ± 11μl respectively which were not significantly affected by co-injection of L-arg (10μmol site^{-1}; 100 ± 11 and 58 ± 5μl respectively). In normal rabbits (n=4), the response to BK (0.5nmol site^{-1}; 50 ± 6μl) was significantly ($p<0.05$) increased to 80 ± 9μl by L-arg (10μmol site^{-1}) whereas the response to HA (13.5nmol site^{-1}; 35 ± 9μl) was not significantly altered (44 ± 12μl). Responses to both mediators were significantly increased by prostaglandin(PG) E_2 (0.3nmol site^{-1}), BK + PGE_2: 140 ± 16μl ($p<0.01$) and HA + PGE_2: 176 ± 36μl ($p<0.01$). In guinea pigs (n=6), responses to HA, both low dose (4.5nmol site^{-1}; 68 ± 5μl) and high dose (13.5nmol site^{-1}; 106 ± 7μl), were significantly ($p<0.01$) reduced by L-arg, 10μmol site^{-1} (38 ± 4 and 60 ± 3μl respectively).

Thus, L-arg increases BK induced PPE in rabbit skin but does not affect responses to antigen or HA. The failure of L-arg to inhibit HA responses in rabbit skin implies that the effect in guinea pigs was not due to chemical interaction between HA and arg.

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233P LACK OF ANTAGONIST ACTION OF L-NAME ON MUSCARINIC (M_3) RECEPTORS IN THE MOUSE ANOCOCCYGEUS

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Much of the evidence supporting the proposal that nitric oxide (NO) is an autonomic neurotransmitter (Rand, 1992) has involved the use of L-arginine analogues, such as L-N^G-nitroarginine methyl ester (L-NAME), which inhibit the enzyme NO synthase (Moore et al., 1990); indeed, the validity of the evidence relies heavily on the selectivity of these inhibitors. Recently, Buxton et al. (1993) have suggested that L-NAME may act as a muscarinic (M_1,M_2 and M_3) receptor antagonist. This caused us some concern, since we have used L-NAME to verify the nitrergic nature of nerve-induced relaxations of the anococcygeus muscle (Gibson et al., 1990; Hobbs & Gibson 1990) and have routinely raised muscle tone by activating smooth muscle muscarinic receptors with carbachol. We have therefore investigated any possible muscarinic antagonist action of L-NAME on the mouse anococcygeus.

Mouse anococcygeus muscles were set up for the recording of isometric responses as described previously (Gibson et al., 1990). Results were calculated as mean ± s.e.

Initial experiments were carried out to determine the subtype of muscarinic receptors in the mouse anococcygeus. Carbachol (1-200μM) produced concentration-related contractions; up to three consecutive curves (20min between curves) could be obtained without significant change in sensitivity (pD_2 values: 5.25±0.04, 5.15±0.04, 5.16±0.08; $P>0.05$). Schild plots of antagonists against carbachol yielded pA_2 values of 7.0 for pirenzipine (slope = 1.0; r = 0.94; n = 12) and 7.9 for hexahydro-sila-difenidol (slope = 1.0; r = 0.98; n = 8). Responses to a submaximally-effective concentration of carbachol (10μM; approx. EC_{75}; 332±48mg) were unchanged (324±50mg) in the presence of 10μM gallamine. These observations indicate the presence of M_3 receptors. Concentration-response curves to carbachol were repeated in the presence of 50μM and 100μM L-NAME; the resultant pD_2 values were 5.71±0.09 for control, 5.61±0.07 with 50μM L-NAME ($P>0.05$), and 5.59±0.06 with 100μM L-NAME ($P>0.05$). The effects of higher concentrations of L-NAME were investigated on responses to 10μM carbachol; these were unaffected ($P>0.05$) by 200μM L-NAME (control 504±31mg; L-NAME 484±41mg) or 500μM L-NAME (control 508±47mg; L-NAME 564±57mg). Field stimulation (10Hz; 10s train every 100s)-induced relaxations were reduced by L-NAME (2-20μM) in a concentration-dependent manner, an effect reversed by 200μM L-arginine; this verified the NOS inhibitory activity of the batch of L-NAME used in the present study.

In conclusion, the muscarinic receptors mediating contraction of the mouse anococcygeus are of the M_3 subtype; L-NAME displays no significant antagonist activity on these receptors.

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234P ROLE OF BRADYKININ AND PLATELET-ACTIVATING FACTOR IN THE IMMEDIATE RELEASE OF NITRIC OXIDE IN RESPONSE TO ENDOTOXIN IN THE ANAESTHETIZED RAT

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Enhanced formation of nitric oxide (NO) contributes to the acute hypotension in circulatory shock elicited by endotoxin (bacterial lipopolysaccharide; LPS) (Thiemermann and Vane, 1990). Bradykinin (BK) and platelet-activating factor (PAF) are possible mediators of this acute activation of the constitutive NO synthase (cNOS), as they are released by LPS into the circulation (Casals-Stenzel et al, 1987; Katori et al, 1989) and release NO from endothelial cells (Fleming *et al*, 1992; Moritoki et al, 1992). Here we investigate the role of NO in the hypotensive action of BK and PAF, and the role of endogenous BK and PAF in the immediate fall in blood pressure caused by LPS. Rats were anaesthetized with sodium thiopental (120 mg/kg i.p.) and mean arterial blood pressure (MAP) and heart rate (HR) were measured via a carotid arterial cannula. The hypotensive effect of BK (10, 30 or 100 μg/kg i.v.) and PAF (0.2 or 1 μg/kg i.v.) was studied in the absence and presence of the NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA; 1 mg/kg/min i.v. infusion for 15 min). LPS (10 mg/kg i.v.) was administered in (i) vehicle-treated rats, (ii) rats pretreated with the PAF antagonist (Casals-Stenzel et al, 1987) WEB 2086 (5 mg/kg 20 min prior to LPS) or (iii) with the B_2 receptor antagonist (Wirth et al, 1991) HOE 140 (2 mg/kg 10 min prior to LPS); MAP was monitored for 30 min. We also studied the effect of the B_1 receptor agonist $^{des}Arg^{10}KD$ (10-300 μg/kg) on MAP and the effect of HOE 140 on the fall in MAP elicited by BK or $^{des}Arg^{10}KD$.

Both BK and PAF caused a dose-dependent immediate fall in MAP. L-NMMA enhanced both the immediate fall in MAP caused by BK (e.g. at 30 μg/kg BK from 29±2 (control) to 55±3 mmHg; $p<0.01$; n=3) and the area under the curve (from 12±1 (control) to 29±2 mmHg min; $p<0.01$; n=3). L-NMMA did not affect the maximum fall in MAP caused by PAF (e.g. at 0.2 μg/kg PAF from 45±3 (control) to 38±6 mmHg; n=6), but significantly shortened the response, thus, reducing the area under the curve (from 51±6 (control) to 20±4 mmHg min; $p<0.01$; n=6). The hypotensive effect of BK was suppressed by HOE 140 by more than 95% (n=5). $^{des}Arg^{10}KD$ (10-100 μg/kg) did not affect MAP, whereas at 300 μg/kg it caused a slight fall in MAP (13±4 mmHg; n=5), which was unaffected by HOE 140 (12±2 mmHg; n=5). LPS (10 mg/kg) caused an immediate (within 5 min) fall in MAP; from 108±4 mmHg to 48±9 mmHg at 15 min (n=5). HOE 140 did not affect this immediate fall in MAP (MAP at 15 min after LPS: 52±12 mmHg, n=3). In contrast, WEB 2086 reduced the acute hypotension caused by LPS (MAP at 15 min: 102±4 mmHg, $p<0.01$, n=6).

Thus, B_2 receptors are predominant in the systemic circulation of the rat; their activation mediates the BK-induced fall in MAP. NO contributes to the hypotensive effect of PAF but not BK *in vivo*. In addition, PAF but not BK contributes to the acute hypotension in response to LPS. Thus, PAF but not BK may be responsible for the acute release of NO by cNOS in response to LPS in the rat. Paradoxically, inhibition of NOS enhances the hypotensive effect of BK. (This work was supported by a grant of Glaxo Group Research Ltd. C.S. is supported by the Lloyds of London Tercentenary Foundation. B.B. is supported by the Heart and Stroke Foundation of Canada and by the FCAR-Quebec).

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235P EVIDENCE THAT REACTIVE HYPERAEMIA IN HUMAN FOREARM SKIN INVOLVES SENSORY NERVES BUT NOT NITRIC OXIDE

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The mechanisms regulating reactive hyperaemia are incompletely understood. To study the involvement of local sensory nerves in this phenomenon, laser-Doppler measurements of skin blood flow were recorded in locally anaesthetised and untreated forearm sites in 8 volunteers following 90, 180, and 360 seconds of arrested forearm blood flow. Reactive hyperaemia increased in magnitude and duration in response to increasing occlusion periods. Maximum post-occlusion flows in the untreated sites of 31 ± 5, 38 ± 6, and 49 ± 5 (mean % flux$\pm$SE) were significantly greater than the 14 ± 3 ($p<0.005$; ANOVA), 20 ± 4 ($p<0.005$), and 25 ± 5 ($p<0.001$) % fluxes seen in the sites pretreated with the anaesthetic cream EMLA (lignocaine/prilocaine 5%). The duration of the hyperaemia was also shortened from 139 ± 26 in the untreated sites to 61 ± 17 seconds (after 360 occlusion, $p<0.02$) in the anaesthetised sites. The anaesthesia did not alter the increase in blood flow induced by intradermally injected calcitonin gene-related peptide (CGRP). Topically-applied capsaicin induced a localised increase in blood flow that was unaffected by anaesthesia and a surrounding flare that was abolished by the treatment (Larkin & Williams 1993). In further experiments hyperaemic responses in 7 volunteers were studied following the intradermal injection of saline vehicle, indomethacin ($1x10^{-9}$moles/site), and the nitric oxide synthesis inhibitor N^G monomethyl-L-arginine (L-NMMA $1x10^{-7}$moles/site). Indomethacin reduced the total blood flow during hyperaemia following 360 second occlusion by 59 ± 8% ($p\leq0.02$), but L-NMMA, which effectively reduced basal skin blood flow, had no effect on hyperaemic responses. The results show that local anaesthesia can significantly inhibit reactive hyperaemia by a mechanism that does not alter the vasodilation induced by exogenous CGRP, or the localised capsaicin-induced release of vasodilators from sensory nerves. The evidence suggests that post-occlusion reactive hyperaemia in human forearm skin does not involve nitric oxide but is part mediated by a local reflex involving sensory nerves and a cyclo-oxygenase product, probably a vasodilator prostaglandin.

Supported by the Wellcome Trust and National Asthma Campaign

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236P THE INFLUENCE OF THE TRIGEMINAL GANGLION ON CRANIAL BLOOD FLOW IN ANAESTHETIZED GUINEA-PIGS

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Stimulation of the trigeminal nerve increases carotid arterial blood flow and decreases carotid vascular resistance in anaesthetized cats, possibly as a result of a vasoactive intestinal polypeptide (VIP)-mediated parasympathetic reflex dilatation of the cranial vasculature (Goadsby & Shelley, 1990; Lambert *et al.*, 1984). It is likely, however, that calcitonin gene-related peptide (CGRP) or substance P, which are localised in, and released from, trigeminal nerve terminals, can also contribute to the effects of trigeminal ganglion stimulation on the cranial vasculature (Goadsby, 1993). In the present study, receptor antagonists for each neuropeptide were used to determine if this was so.

Adult male guinea-pigs (300-450g) were anaesthetized (i.p.) with ketamine ($50mgkg^{-1}$) and pentobarbitone ($25mgkg^{-1}$) and the right carotid artery was cannulated for measurement of arterial blood pressure. Carotid arterial blood flow was measured with a Doppler flow probe placed around the left common carotid artery and carotid vascular resistance was monitored continuously using a peripheral resistance meter. Bilateral burr holes were drilled in the skull above the trigeminal ganglia and a bipolar stimulating electrode was lowered through the brain by a micromanipulator into each trigeminal ganglion.

Electrical stimulation (0.5-1.5mA, 1.0ms, 5Hz, 60s) of the left trigeminal ganglion reduced arterial blood pressure and left carotid vascular resistance with no consistent alteration in left carotid blood flow (changes from resting levels of -24.1 ± 4.8% (n=16), -7.5 ± 3.8% (n=16) and -8.5 ± 8.2% (n=16) respectively at 0.5mA). However, in guinea-pigs pretreated with guanethidine ($30mgkg^{-1}$ s.c., 24h prior to experiments), trigeminal ganglion stimulation (0.5-1.5mA, 1.0ms, 5Hz, 60s) produced little effect on blood pressure, but increased carotid arterial blood flow and decreased carotid vascular resistance (changes from resting levels of -3.1 ± 6.5, 10.9 ± 6.6 and -20.7 ± 4.2% respectively at 0.5mA stimulation (n=6)). In guanethidine-pretreated animals, the VIP receptor antagonist, [p-Cl-D-Phe^6,Leu^{17}]-VIP ($50\mu gkg^{-1}$ i.v.), significantly ($p<0.05$, Student's t-test) attenuated the increase in carotid blood flow and decrease in carotid vascular resistance evoked by trigeminal ganglion stimulation. Responses were, however, unaffected by the tachykinin NK_1 receptor antagonists, GR82334 ($0.3mgkg^{-1}$ i.v.; Hagan *et al.*, 1991) and CP-99,994 ($0.4mgkg^{-1}$ i.v.; Desai *et al.*, 1992), or by the CGRP receptor antagonist, $CGRP_{8-37}$ ($0.9mgkg^{-1}$ i.v.), at doses which block the vasodepressor effects of either substance P methyl ester ($0.04\mu gkg^{-1}$ i.v.) or human αCGRP ($0.4\mu gkg^{-1}$ i.v.), respectively.

It is concluded that in guanethidine-pretreated guinea-pigs, electrical stimulation of the trigeminal ganglion increases carotid blood flow and produces an accompanying decrease in carotid vascular resistance, consistent with dilatation of cranial blood vessels. The transmitter mediating this effect is most likely to be VIP. The study also suggests that trigeminal ganglion stimulation in the absence of guanethidine inhibits a sympathetically-mediated cranial vascular tone.

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237P ANTAGONISM OF HUMAN α-CGRP RELAXATION BY HUMAN $\alpha CGRP_{8-37}$ IN RAT INTERNAL ANAL SPHINCTER

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Recent evidence has shown that human α-calcitonin gene related peptide (hα CGRP) inhibition of the spontaneous tone of the oppossum internal anal sphincter is antagonised by hα $CGRP_{8-37}$ (pA_2 7.8; Chakder & Rattan, 1991). However, consistently lower values have been obtained for hα $CGRP_{8-37}$ in a number of rat tissues but the internal anal spincter has not been used. Whether the lower potency of hα $CGRP_{8-37}$ reflects the differing tissue or different species is not clear. Therefore, the aim of the present experiments was to study hα $CGRP_{8-37}$ in the rat internal anal sphincter and vas deferens to compare with the value reported in the oppossum internal anal sphincter.

Strips of the internal anal sphincter and prostatic half of the vas deferens from male Sprague Dawley rats (300-400g) were set up in Krebs solution at 34°C and 37°C respectively, bubbled with 95%O_2/5%CO_2 and placed under 0.5 g resting tension. The sphincter strips developed spontaneous tone. The vas deferens was field stimulated (60V, 1ms, 0.2 Hz) via platinum wire electrodes. Responses were measured isometrically and are given as mean±s.e. mean.

In the rat internal anal sphincter, hα CGRP (10^{-10}-$3x10^{-6}$M) and vasoactive intestinal polypeptide (VIP; 10^{-11}-10^{-7}M) induced a dose dependent inhibition of spontaneous tone (EC_{50} $1.9 \pm 0.4x10^{-9}$M, $6.4 \pm 1.0x10^{-10}$M, respectively), with a maximum fall in tension of 100%. The hα CGRP responses were antagonised by hα $CGRP_{8-37}$ (10^{-5}M), inducing a parallel shift to the right, without a reduction in the maximum response (pK_B 5.7). VIP responses, however, did not change in the presence of hα $CGRP_{8-37}$ (up to 10^{-5}M), showing selectivity of the antagonist. In the rat vas deferens hα CGRP dose dependently inhibited twitch responses to field stimulation (EC_{50} $6.6\pm0.7x10^{-9}$M). This was competitively antagonised by hα $CGRP_{8-37}$ (pK_B 5.9 at 10^{-5}M).

In the internal anal sphincter from the rat, hα CGRP and VIP were around 30 and 300 times more potent respectively than in the same tissue from the oppossum (Chakder & Rattan, 1990; 1992). The hα CGRP responses were inhibited by hα $CGRP_{8-37}$ in both the rat and the oppossum sphincter but in the rat tissue the antagonist effect was much weaker. In addition, the value in the rat sphincter was similar to that for the rat vas deferens (pK_B 5.7, 5.9 respectively; vas deferens pA_2 6.2, Quirion *et al.*, 1992). Therefore, the similar pK_B values for the internal anal sphincter and the vas deferens in the rat, coupled with the widely different value in the oppossum sphincter (Chakder & Rattan, 1991), suggest a species rather than a tissue difference in the CGRP receptors.

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238P COMPARISON OF RESPONSES TO CALCITONIN GENE-RELATED PEPTIDE IN CULTURED EPITHELIA AND RAT COLON MUCOSA

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Calcitonin gene-related peptide (CGRP) stimulates complex biphasic changes in ion transport across voltage-clamped mucosal preparations of rat colon (Cox et al.1989). In the presence of tetrodotoxin (TTX) only secretory responses were observed with basolateral application of α-CGRP (1-100nM). To investigate CGRP effects upon epithelia in the absence of other cell types, we have used two stable cell lines namely, HCA-7 cells which were established from a primary human adenocarcinoma (Kirkland, 1985) and colony-29 (Col-29) cells, a subpopulation isolated from the parent cell line. Both cell lines secrete chloride in response to various agonists (MacVinish et al. 1993) and the aim of this study was to establish the location and type of CGRP receptor expressed by these epithelia and compare them with those present in mucosal preparations of rat descending colon.

The conditions for cell culture were as described in detail by Cuthbert et al. (1987). Cells ($4-6x10^5$ in 100µl) were seeded onto collagen coated millipore filters and reached confluence in 7-10days, covering an area of $0.2cm^2$. Each filter or mucosal sheet of rat colon (Cox et al. 1989) was placed between two halves of an Ussing chamber, and bathed in oxygenated Krebs-Henseleit solution at 37°C. All epithelial preparations were voltage-clamped at zero potential (WP Instruments) and the resultant short circuit current (SCC) recorded continuously. Peptides (from Peninsula Labs Inc) were added to either the basolateral or apical surface once a stable basal SCC was achieved. EC_{50} values were calculated using the curve fitting programme, Graphpad.

The basal SCC of HCA-7 cells was $8.5\pm1.5\mu A.cm^{-2}$ (n=33) and Col-29 was $8.5\pm0.5\mu A.cm^{-2}$ (n=47). Maximally effective concentrations of rat α- and β-CGRP in HCA-7 cells were 3nM (increased SCC by $20.5\pm5.5\mu A.cm^{-2}$, n=3) and 1nM (increased SCC by $16.5\pm3.5\mu A.cm^{-2}$, n=4) respectively. In Col-29 cells 30nM rat α-CGRP and 100nM β-CGRP stimulated maximal increases in SCC of $7.6\pm1.4\mu A.cm^{-2}$ (n=12) and $7.5\pm0.9\mu A.cm^{-2}$ (n=7). EC_{50} values for α- and β-CGRP were 0.6 and 0.5nM in HCA-7, and 4.4 and 9.8nM in Col-29 cells respectively, while in colon mucosa (+TTX,100nM) the α-CGRP EC_{50} was 35.6nM. Apical additions of 3nM α-CGRP to HCA-7, or 30nM to Col-29 cells increased SCC by only $1.0\pm0.2\mu A.cm^{-2}$ (n=4) and $2.7\pm0.4\mu A.cm^{-2}$ (n=6) respectively. No change in SCC was seen with apical peptide additions to rat colon mucosa. Pretreatment with the human sequence, (8-37)CGRP (up to 1µM) did not significantly alter α-CGRP responses in either HCA-7, Col-29 or in colon mucosa. We conclude that epithelial CGRP receptors are expressed predominantly on the basolateral surface, and whether in normal rat colon or adenocarcinoma cells they exhibit CGRP type II-like receptor characteristics, since responses were not sensitive to the fragment (8-37)CGRP.

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239P STRUCTURE-ACTIVITY RELATIONSHIP OF ANALOGUES OF THE ANTAGONIST hαCGRP$_{8-37}$ IN RAT PROSTATIC VAS DEFERENS

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CGRP inhibits the twitch responses to field stimulation of the rat vas deferens (Ohhashi & Jacobowitz, 1985). This effect is antagonised by the fragment human α CGRP$_{8-37}$ (hα CGRP$_{8-37}$)(Dennis *et al.*, 1990). The aim of these studies was to investigate the significance of the C-terminal amino acid phenylalanine and to investigate the predicted existence of a ß-turn in the molecule between amino acid residue 17 to 21 (Hubbard *et al.*, 1991). The structure-activity relationship of hα CGRP$_{8-37}$ analogues was examined by substitutions in position 37 and in the bend region. The effect of these fragments was assayed against hα CGRP using the rat prostatic vas deferens.

Male Sprague Dawley rats (300-400g) were stunned and killed by cervical dislocation. The vas deferens was removed, isolated from associated blood vessels and membranes, bisected and the prostatic portion set up in Krebs solution at 37° C and bubbled with 95%O_2/5%CO_2. After the tissue was placed under 0.5g resting tension and allowed to equilibrate for 1 hour the vas deferens was field stimulated (60V, 1ms, 0.2Hz) by parallel platinum wire electrodes. Isometric tension was recorded by a polygraph. Cumulative concentration response curves to hα CGRP (10^{-10}- 10^{-6}M) were obtained in the absence and presence of hα CGRP$_{8-37}$ and its analogues (preincubated for 20 minutes).

Inhibition of twitch responses by hα CGRP (EC_{50} 6.6±0.67×10^{-9}M, mean±s.e.mean, n=20) was antagonised by hα CGRP$_{8-37}$ (10^{-5}M), with no depression of the maximum response (pK_B 5.9). The substitution of the C-terminal amino acid phenylalanine (position 37) by alanine abolished antagonist activity (at 10^{-5}M). Similar, the substitution for leucine by the bend biasing amino acid proline at position 16 (i.e. before the suggested β-turn) abolished antagonism, whereas with alanine in the same position, the activity of the fragment remained (pK_B 5.6 at 10^{-5}M). When proline was substituted for serine in position 19 (i.e. within the proposed β-turn) the antagonist activity was unchanged from the original fragment (pK_B 5.7 at 10^{-5}M).

The C-terminal amino acid phenylalanine appears to be essential for the biological activity of the molecule as the alanine substituted analogue was not an antagonist. This suggests that the terminal amino acid may have a role in receptor affinity. Induction of a bend by proline (position 16, i.e. before the ß-bend), abolished antagonism whereas within the bend region proline substitution did not alter biological activity. Therefore, these results are consistent with a β-bend between amino acid residues 17 to 21 and suggest this may be an important structure in the conformation of the molecule for binding to the CGRP receptor.

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240P BIOCHEMICAL CHARACTERISATION OF SR48692, A SELECTIVE NON-PEPTIDE ANTAGONIST OF THE NEUROTENSIN RECEPTOR

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SR48692 has recently been characterized as a competitive inhibitor of neurotensin (NT) receptors in different tissues from various species (Gully et al, 1993). The aim of the present work was to further investigate the effects of SR48692 on functional events linked to NT receptor activation, using two cellular models, HT29 and N1E115. Stimulation of NT receptors in the human colonic carcinoma HT29 led to an increase in inositol phosphates (IPs) without affecting cyclic nucleotide levels (Amar et al, 1986). In the mouse neuroblastoma N1E115, NT was found to increase IPs (Snider et al, 1986) and also cGMP levels (Gilbert et al, 1986)

HT29 cells were grown in 6 well-plates and equilibrated for 18h in culture medium, containing 1µCi ml^{-1} of myo[^{3}H]-inositol. After 20 min incubation with 20 mM LiCl, cells were stimulated for 30 min with NT in the absence or presence of SR48692. [^{3}H]-inositol 1-phosphate (IP1) was isolated by anion exchange chromatography with Dowex 1-X8. In N1E115 cells differentiated in 24 wells, IP1 was measured as described above. For cGMP assay, differentiated cells were incubated for 4h in normal culture medium before use. Cells were then incubated for 5 min in the presence or absence of SR48692 and stimulated 20 s with NT. cGMP content was measured with the radioimmunoassay kit from Amersham. For comparison, binding assays were conducted on intact cells, using ^{125}I-NT as ligand, according to Gully et al (1993).

NT stimulated the accumulation of IP1 in HT29 and N1E115 with an EC_{50} of 3.5 ± 1.8 x 10^{-9}M and 4.6 ± 6.8 x 10^{-10}M, respectively. NT also stimulated the formation of cGMP in N1E115 cells with an EC_{50} of 0.47 ± 3.5 x 10^{-9}M. In the N1E115, SR48692 antagonized IP1 accumulation and cGMP production induced by 3 x 10^{-10}M NT with an IC_{50} of 2.1 ± 2.1 x 10^{-9}M and 2.5 ± 1.73 x 10^{-9}M, respectively. In HT29 cells, SR48692 antagonized 10^{-8}M NT-induced IP1 accumulation with an IC_{50} of 2.8 ± 1.3 x 10^{-8}M. The competitive antagonism obtained for IP1 formation revealed pA2 values of 8.7 ± 0.2 for HT29 and 10.1 ± 0.2 for N1E115 cells. These differences could be accounted for by a more efficient coupling in N1E115 vs HT29 cells, since binding experiments revealed similar affinity of SR48692 for NT receptor in these two cell lines (IC_{50} = 15.3 ± 7.0 x 10^{-9}M and 20.4 ± 9.5 x 10^{-9}M, respectively).
In conclusion, SR48692 appears to be a potent antagonist of the signal transduction events triggered by NT receptor activation in both peripheral and central cell systems.

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241P NICOTINAMIDE, AN INHIBITOR OF MONO(ADP-RIBOSYL)TRANSFERASE, SELECTIVELY INCREASES THE ABUNDANCE OF Gsα BUT NOT OTHER G-PROTEIN SUBUNITS IN NG108-15 CELLS

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Eukaryotic mono(ADP-ribosyl)transferases are a group of enzymes whose cellular substrates and functions are poorly understood. This contrasts with the prokaryotic mono(ADP-ribosyl)transferases whose substrates include Gsα, Giα and EF-2. There is now evidence that an endogenous enzyme mono(ADP-ribosyl)ates Gsα in NG108-15 cells, and modifies its function (Donnelly et al 1992). Inhibition of the endogenous mono(ADP-ribosyl)transferase by nicotinamide leads to an increase in the abundance of Gsα. We have now examined whether treatment with this inhibitor affects the abundance of other G-protein subunits, or whether this effect is selective. We have also investigated whether Giα is a substrate for mono(ADP-ribosyl)transferase in these cells.

NG108-15 cells were exposed for 17 h to selected concentrations of nicotinamide in the range 0.5-50 mM. The cells were harvested and membranes prepared. The abundance of the various G-protein sub-units was measured using immunoblotting with specific antibodies. Membranes from cells which had been treated in the absence or presence of 50 mM nicotinamide were labelled with [α^{32}P]-NAD in the presence of either cholera or pertussis toxin and the [^{32}P]ADP-ribosylated proteins determined by autoradiography. The possibility that Giα may be a substrate for mono(ADP-ribosyl)transferase was examined by labelling membranes with [α^{32}P]-NAD, and immunoprecipiation of labelled proteins with an anti-Giα antibody (Anderson and Blobel 1983).

Treatment of cells with nicotinamide in the range 0.5 to 10 mM had no effect on the abundance of immunoreactive Gsα, while exposure of the cells to 50 mM nicotinamide resulted in a significant ($p<0.05$) increase (208.2 ± 33.7%, n=10) above the control. This treatment also resulted in a significant ($p<0.05$) increase in cholera substrate in these membranes (240.8 ± 54.2%, n=6). Immunoblotting of the membranes with antibodies against Giα, Goα or Gß revealed no significant difference in the abundance of each subunit when compared to the control. Furthermore, the abundance of pertussis toxin substrate was also unaltered by this treatment. Immunoprecipitation with anti-Gsα antibody of ^{32}P-labelled membranes confirmed our earlier result that Gsα is ADP-ribosylated (Donnelly et al 1992), however immunoprecipitation with an anti-Giα antibody revealed no evidence of ADP-ribosylated Giα.

The inhibition of mono(ADP-ribosyl)transferase with nicotinamide increased selectively the abundance of Gsα, but not other G-protein subunits. We conclude that Giα is not a substrate for mono(ADP-ribosyl)transferase in NG108-15 cells.

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242P THE EFFECTS OF TRIHYDROXYPHENYL-ALANINE AND PYRIDOXAL PHOSPHATE ON THE INHIBITION OF CHICK BONE AND CARTILAGE LYSYL OXIDASE BY β-AMINOPROPIONITRILE

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Lysyl oxidase (EC 1.4.3.13) is a semicarbazide-sensitive amine oxidase that oxidatively deaminates peptidyl lysine residues in collagen and elastin as an early stage in their cross-linking. The enzyme is irreversibly inhibited by β-aminopropionitrile (BAPN), a known lathyrogen, *in vitro* and *in vivo*, while the inhibition by semicarbazide (SC) is reversible (see Levene *et al.*, 1992). The enzyme requires copper and another co-factor whose identity is still uncertain. Historically, pyridoxal phosphate (PLP) has been favoured but, recently, trihydroxyphenylalanine (TOPA) has been identified as the co-factor of some other semicarbazide-sensitive amine oxidases (Janes *et al.*, 1990), raising the possibility that it could serve the same function in lysyl oxidase.

Lysyl oxidase was extracted from the long bones and cartilages of 16-day chick embryos and the peptidyl-[^{3}H]-lysine substrate prepared from chick aorta. Enzyme activity, was estimated, as d.p.m. mg^{-1} protein, from the amount of [^{3}H]-water produced from lysine deamination after 2 h incubation at 37° C with and without inhibitors and putative co-factors. Specific activity of the substrate cannot be determined since the number of available labelled peptidyl-lysine groups is unknown and no satisfactory defined substrate has yet been discovered. The [^{3}H]-lysine substrate was usable up to 3 weeks when net enzyme activities became too low due to isotope exchange and a rise in background radioactivity. For a detailed description of the methods, see Levene *et al.* (1992). Each result is the mean of triplicate assays on pooled enzyme and substrate from 36 embryos.

Control activities were measured at 0, 1 and 2 weeks after preparation and were found to be 595, 417 and 176 d.p.m. mg^{-1} protein, indicative of the progressive decline in enzyme activity. Control experiments with either TOPA or PLP added to uninhibited enzyme showed that neither agent significantly affected activity. BAPN (0.4 mM) totally inactivated the lysyl oxidase activity after 1 h incubation together with substrate. At this time either TOPA (0.4 mM) or PLP (0.4 mM) was added followed by a further 1 h incubation. TOPA more than fully reversed the inhibition caused by BAPN and resulted in a final enzyme activity of 186 ± 62% (s.e.r., n = 3) when compared with uninhibited control. PLP had no effect and the enzyme remained totally inhibited. When, the reversible inhibitor, SC (0.4 mM) was used, enzyme activity was again reduced to zero. In this case both TOPA and PLP were able to reverse the inhibition, resulting in enzyme activities of 243% and 206% of control, respectively (triplicate observations on pooled samples). No spontaneous recovery of enzyme activity was observed nor did the activity of uninhibited enzyme increase under these experimental conditions.

This present study shows for the first time that TOPA restores the activity of BAPN-inhibited chick embryo lysyl oxidase *in vitro* to levels beyond even that of control samples while PLP was quite ineffective. Until now, it has been impossible to reactivate BAPN-inhibited lysyl oxidase. Although these results do not provide direct evidence that TOPA is the physiological non-copper co-factor of chick lysyl oxidase, the fact that it alone reverses the inhibition of enzyme activity by the powerful lathyrogen, BAPN, suggests that its claim cannot be ignored. The increase in enzyme activity to above control values caused by the addition of TOPA to BAPN-inhibited enzyme requires further investigation as does the increase seen with TOPA and PLP in the SC-inhibited enzyme.

This work was supported by the Wellcome Trust.

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243P OXIDATION OF CYTOCHROME C BY THE J774 MACROPHAGE CELL LINE

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Polymorphonuclear leucocytes are known to release superoxide anion following stimulation by phorbol 12-myristate 13-acetate (PMA) and f-met-leu-phe (De Kimpe *et al.*; 1993, McCall *et al.*, 1989). Macrophages too are capable of producing free radical species when activated, and these are vital in the killing of invading organisms. The aim of this study was to assess superoxide production by J774 macrophages by measuring the reduction of ferricytochrome C (De Kimpe *et al.*, 1993).

J774 cells were grown at 37°C in HEPES-buffered Dulbecco's Modified Eagle's Medium (DMEM) in sterile siliconised Techne stirrer bottles. On the day of use, the cells were washed with saline and suspended at a density of 10^6 cells ml^{-1} in HEPES-buffered Krebs. Superoxide generation was detected by measuring the absorbance of ferricytochrome C (10μM) at 550 nm in the presence of catalase (100 U ml^{-1}) to prevent reoxidation by hydrogen peroxide.

Resting J774 cells, and cells stimulated with PMA (10^{-7} M) or lipopolysaccharide (100 ng ml^{-1}) failed to reduce ferricytochrome C. Hypoxanthine (HX, 0.2 mM) and xanthine oxidase (XO, 20 mU ml^{-1}) produced an increase in absorbance of around 17-fold on addition to ferricytochrome C (10 μM), indicative of superoxide production. This effect, however, was completely abolished in the presence of J774 cells (10^6 cells ml^{-1}), suggesting that the cells might have generated a powerful oxidant. This was investigated by preparing the reduced form of cytochrome C (10 μM) using sodium dithionite (100 μM), in order to detect the presence of an oxidising species. Addition of J774 cells produced almost complete oxidation (85 ± 2 %) of the reduced cytochrome C within 2 hours. Incubation of J774 cells with reduced cytochrome C (10 μM) resulted in 35 ± 4 % oxidation after 30 min. Separating the cells from cytochrome C at this point prevented further oxidation. Oxidation of cytochrome C by the cells was unaffected by superoxide dismutase (100 U ml^{-1}), catalase (10 mM), the inhibitor of nitric oxide synthase, N^G-monomethyl-L-arginine (500 μM), or the hydroxyl radical scavengers, dimethylthiourea (1mM) and mannitol (10mM).

Our findings demonstrate that J774 cells are able to produce a labile oxidising species that opposes the reduction of cytochrome C by superoxide anion. We are unable to identify the species at this point, but our data suggest that it is unlikely to be peroxynitrite, hydrogen peroxide or hydroxyl radical.

This work was supported by the British Heart Foundation.

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244P REGIO- AND ENANTIOSELECTIVE METABOLISM OF METOPROLOL BY TWO HUMAN cDNA-DERIVED CYP2D6 PROTEINS

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Two human *CYP2D6* cDNAs containing functional coding sequences have been isolated independently by Gonzalez (Gonzalez *et al.*, 1988) and by us (Wolf, unpublished data). The coding sequences of the two cDNAs are identical except for two base differences at 1120 (G vs A) and 1488 (A vs C) in the cDNA isolated by us. The latter base difference is silent but G→A at 1120 results in an amino acid substitution from valine to methionine in the CYP2D6 encoded by the cDNA isolated by Gonzalez.

Heterologous expression of the two cDNAs in yeast revealed similar levels of expression of the recombinant P450 protein, as determined by CO-difference spectroscopy (40 ± 33 and 37 ± 36 SD pmol/mg protein for the cDNAs of Gonzalez and Wolf, respectively). A Soret absorption maximum at 448nm was observed for each protein and no P420 was detected.

Each form of CYP2D6 catalyzed both the O-demethylation (ODM) and α-hydroxylation (α-OH) of metoprolol. Each showed identical metoprolol enantioselectivity, i.e. α-OH of R-(+) and S-(-) metoprolol was catalyzed at the same rate while ODM was faster with the R-(+) enantiomer. These findings are consistent with observations with human liver microsomes prepared from extensive metabolizers (EM) (Otton *et al.* 1988). However, the ratio of ODM to α-OH with the two forms of CYP2D6 was significantly different ($P < 0.005$), irrespective of whether the racemate (*rac*) or enantiomers of metoprolol were used as substrate. Thus, with *rac*-metoprolol, an ODM to α-OH ratio of 2:1 was observed with the CYP2D6 derived from the cDNA of Gonzalez, while a ratio of 4:1 was observed with the protein derived from the cDNA isolated by Wolf.

The metabolism of *rac*-metoprolol by human liver microsomes prepared from extensive metabolizers gave an ODM to α-OH ratio of 5.5:1. ODM in EM livers is attributed to two P450s, the CYP2D6 contribution amounting to approximately 70% as determined by quinidine inhibition (Otton *et al.*, 1988). Extrapolation from these human liver data would, therefore, predict an ODM to α-OH ratio of approximately 4:1 with human CYP2D6 expressed heterologously in yeast, as observed with the enzyme encoded by the cDNA isolated by us.

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245P SURFACTANT POTENTIATION OF YEAST SENSITIVITY TO ANTIBIOTICS

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Yeast permeability to small molecules can be modified by supplementation of growth media with surfactants. For example, uptake of fluorescein diacetate can be markedly altered by exposure to the non-ionic surfactant, Pluronic F-68 (King *et al.*, 1991). This may be due to transmembrane "pores" caused by Pluronic-induced molecular aggregates within the cell membrane (King *et al.*, 1991). This is consistent with results with cultured animal cells showing increased 2-deoxyglucose uptake and amino acid incorporation with Pluronic F-68 (Cawrse *et al.*, 1991). This raises the question of whether surfactants can modify microbial responses to antibiotics, by altering cell permeability. We have therefore examined the effects of Pluronic F-68 and its fractions or Triton X-100 on yeast sensitivity to cycloheximide (CYC) or amphotericin B (AMB). Starter cultures (s.c.) of *Saccharomyces cerevisiae* (strain X 2180 1B) were grown overnight in YP medium at 30°C. Experimental cultures (0.12 ml of s.c. in 12.0 ml medium) of *S. cerevisiae* were grown for up to 24 h either in YP medium alone or medium containing 1.0% (w/v) of either (i) commercial grade (COMM) Pluronic F-68 (ICI, U.K.); (ii) a silica-purified Pluronic F-68 fraction (FRAC; Bentley *et al.*, 1989); or (iii) Triton X-100 (Sigma, U.K.). In test cultures, 0.1 µg/ml CYC (Sigma, U.K.) or 50 µg/ml AMB (Gibco, U.K.) was added after 250 min; these are sub-lethal doses of these drugs in yeast. Culture growth over the subsequent 24 h was assessed by optical density (O.D.) at 600 nm.

Yeast growth with 1.0% (w/v) of COMM or FRAC Pluronic F-68 was similar to control. In contrast, 1.0% (v/v) Triton X-100 slowed the onset of the logarithmic growth phase such that after 600 min, the mean O.D. was 19-55% lower ($P<0.05$) than control. Addition of 0.1 µg/ml CYC to cultures after 250 min of growth reduced the mean ($\pm$ s.e.m.; n = 3 throughout) O.D. after a further 200 min to 0.99 $\pm$ 0.09 compared to 1.21 $\pm$ 0.06 in control ($P<0.05$). Cultures grown with COMM Pluronic F-68 showed a mean O.D. of 0.78 $\pm$ 0.03 at 200 min after addition of CYC, with no further difference when FRAC Pluronic was used. Addition of 0.1 µg/ml CYC to yeast cultures grown for 250 min with 1.0% (v/v) Triton X-100 reduced the mean O.D. after a further 200 min of growth to 0.65 $\pm$ 0.05 compared to 1.08 $\pm$ 0.06 for cells treated with CYC alone ($P<0.05$). Addition of 50 µg/ml AMB to cultures after 250 min of growth without surfactants reduced the mean O.D. after a further 200 min to 0.88 $\pm$ 0.05 compared to 1.36 $\pm$ 0.04 in controls ($P<0.01$). Cultures grown with COMM Pluronic F-68 showed a mean O.D. of 0.58 $\pm$ 0.07 at 200 min after addition of AMB. There was no significant difference between the effects of COMM or FRAC Pluronic. Addition of 50 µg/ml AMB to yeast cultures grown for 250 min with 1.0% (v/v) Triton X-100 reduced the mean O.D. after a further 200 min to 0.44 $\pm$ 0.03 compared to 0.83 $\pm$ 0.05 for cells treated with AMB alone ($P<0.01$). These results show that growth of *S. cerevisiae* cultures is partially inhibited by 0.1 µg/ml CYC or 50 µg/ml AMB. Such use of sub-lethal doses of antibiotics provides a convenient means for probing surfactant effects on cell permeability. Previous work (King *et al.*, 1991) suggests that Pluronic F-68 may induce transmembrane pore formation which may facilitate antibiotic uptake into yeast. Triton X-100 was more effective than Pluronic F-68 in enhancing yeast sensitivity to antibiotics and this may reflect the well documented cell permeabilizing properties of this compound (Laouar *et al.*, 1992).

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246P TRANSDERMAL IONTOPHORESIS: ROLE OF THE STRATUM CORNEUM AND EPIDERMIS IN SOLUTE TRANSPORT

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The stratum corneum and epidermis (avascular layer) have long been considered to be the major rate limiting barrier to passive transdermal drug absorption. Transdermal iontophoresis, applying an electrical potential gradient across the skin via two electrodes, has been shown to produce absorption rates of charged solutes comparable to passive absorption following removal of the avascular layer (Singh & Roberts, 1993). The aim of this study was to determine the role of the avascular layer barrier in iontophoretic transdermal solute transport.

Male Wistar rats were anaesthetised (sodium pentobarbitone, 60mg/kg i.p.) and two glass diffusion cells (donor and receptor) fixed to the depilated (Nair™ cream) abdominal skin. The avascular layer of the skin beneath the donor cell (containing the anode electrode (silver), 2ml 50mM Hepes buffer pH 7.4 plus one of the following radiolabelled monovalent cationic solutes of differing molecular weight: sodium (A), ethanolamine (B), phenylethylamine (C), glucosamine (D) and lidocaine (E)) was either removed with a hand held dermatome to expose the underlying dermis or left intact (normal). The second (receptor) cell containing the cathode electrode (silver chloride) and 3ml 20mM isotonic Hepes buffer pH 7.4 was always fixed to normal skin. The iontophoretic current (0.38mA/cm^2) was applied for 120 min whilst monitoring solute loss from the donor cell by frequent sampling of the cell contents to provide a measure of transdermal clearance. Solute permeability coefficients (PCiont) were determined by: transdermal clearance (ml/hr) / surface area (cm^2).

The present study showed that following the removal of the avascular layer in dermis exposed animals the rate of iontophoretic delivery of the various cations studied was significantly slower, with the exception of glucosamine, than that through normal skin (Table 1).

Table 1. PCiont of monovalent cationic solutes delivered by iontophoresis in normal and dermis exposed animals.

Solute		A	B	C	D	E
PCiont	Normal	0.91±0.12	0.33±0.01	0.31±0.02	0.10±0.02	0.25±0.03
(ml/hr/cm^2)	Dermis Exposed	0.14±0.02**	0.16±0.02**	0.18±0.02**	0.12±0.02	0.14±0.03*

Mean±s.e.m, n=3; *P<0.05, **P<0.01 (Student's *t* test).

The depth of penetration of solutes into deeper skin and muscle tissue below the application site was similar in both normal and dermis exposed animals. However, although the concentrations of solute in the skin layer (0-2mm), expressed as a fraction of the applied concentration, were identical, for example for ethanolamine 0.79±0.24 and 0.78±0.13, in normal and dermis exposed animals respectively, the concentration in the avascular layer of normal animals was 1.6 fold higher than in the underlying skin layer.

We conclude that the higher permeability coefficients of solutes as a result of iontophoresis through normal skin than exposed dermis reflects the function of the avascular layer as a form of temporary reservoir into which solutes can relatively quickly accumulate. Similarity in solute concentrations beneath the application sites in tissues from both normal and dermis exposed animals suggests that the avascular layer also provides an effective conductance route for the extra solute ions absorbed to migrate laterally toward the opposite electrode.

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247P AIRWAY OBSTRUCTION DURING A DELAYED-TYPE HYPERSENSITIVITY REACTION IS NOT ASSOCIATED WITH INCREASED AIRWAY REACTIVITY IN THE GUINEA-PIG

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It is widely considered that pulmonary inflammation is the primary determinant of airway hyperreactivity (British Thoracic Society, 1993). Indeed, it has previously been reported that inflammation of the lung during a delayed-type hypersensitivity (DTH) reaction was associated with increased airway reactivity in the mouse (Van-Loveren et al., 1991). The present study has sought to determine the effect upon pulmonary mechanics of a DTH reaction in the guinea-pig.

Adult male guinea-pigs (Dunkin-Hartley, 500-600 g) were sensitised by subcutaneous injection of bovine serum albumin (BSA) conjugated with arsanilic acid (AS), (10 $\mu g\ kg^{-1}$) in Freund's complete adjuvant (1 ml). After 14 days, animals responded to intracutaneous injection of BSA-AS (1 and 10 $\mu g\ site^{-1}$) with indurated reactions of delayed onset that were maximal 24 h following injection of BSA-AS. 3 days following resolution of the skin reaction, sensitised guinea-pigs were exposed to nebulised aerosols of either physiological saline (0.9% for 30 min) or BSA-AS (1 mg ml^{-1} for 30 min). 24 h later, basal airway resistance (R_L $cmH_2O\ l^{-1}\ sec^{-1}$) and dynamic compliance (C_{dyn} ml cmH_2O^{-1}) as well as airway reactivity (maximal increase in R_L, mean ± sem) to histamine (Hist, 0.56,1.0,1.8,3.2 $\mu g\ kg^{-1}$ i.v.) or acetylcholine (ACh, 5.6,10,18 $\mu g\ kg^{-1}$ i.v.) was determined (Chapman et al., 1992). Following (24 h) exposure to saline, neither R_L (96.7 ± 4.0 n=20) nor C_{dyn} (0.9± 0.05, n=20) differed from the normal range for this preparation (R_L 92.5 ± 0.95 and C_{dyn} 0.99 ± 0.01, n=300) and airway reactivity to Hist was 23.7 ± 4.9, 84.5 ± 28.9, 268.3 ± 74.8, 629.6 ± 89.3. and to ACh was 83.7 ± 22, 333.0 ± 95.6, 687 ± 61.9 respectively. However, following inhalation of BSA-AS there was significant ($p<0.001$) reduction in C_{dyn} (0.38 ± 0.05, n=20) and slight, but significant ($p=<0.01$), increased R_L (143 ± 9.1, n=20). Despite these changes in pulmonary function, airway reactivity to Hist (52.1 ± 8.7**, 159.3 ± 32.4, 510.4 ± 101.9, 810.6 ± 126.3) and to ACh (98.9 ± 27.8, 440.6 ± 106.8, 937.9 ± 86.6) was not significantly different from that in animals exposed to saline, excepting for the lowest dose of Hist (** $p<0.01$) .

These data demonstrate that a DTH reaction in the guinea-pig lung is characterised by a significant decrease in pulmonary compliance and to a lesser extent increased airway resistance; changes that were not accompanied by increased airway reactivity to intravenously administered histamine or acetylcholine. These findings are not consistent with the conclusions of Van Loveren et al., 1991 and question the hypothesis that airway hyperreactivity is a consequence of airway obstruction due to pulmonary inflammation.

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248P ROLE OF THE LEUKOCYTE ADHESION MOLECULE CD11b IN COMPLEMENT-INDUCED NEUTROPHIL SEQUESTRATION AND ACTIVATION IN ACUTE LUNG INJURY IN MICE

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It has been demonstrated that systemic activation of the complement system by i.v. injection of cobra venom factor (CVF) can result in acute lung injury in rats and mice (Till *et al*, 1982: Tvedten *et al*, 1985). The injury is characterised by increased vascular permeability and is accompanied by extensive retention of leukocytes in pulmonary capillaries. Neutrophil depletion results in an alleviation of the injury, thus demonstrating the requirement for neutrophils in the induction of permeability changes. Furthermore, Mulligan *et al* (1993) has reported that the blockade of the leukocyte CD11b adhesion molecule in rats resulted in a reduction of permeability induced by CVF. We have previously described a model of acute lung injury in the mouse in which leukocyte adhesion via CD11b is involved in mediating increases in vascular permeability induced by combined LPS and zymosan treatment (Miotla *et al*, 1993). In the present study, we have investigated the role of CD11b in mediating lung injury induced by CVF or zymosan in the mouse.

All experiments were carried out in BALB/c mice (18-20g). The animals were anaesthetised and injected with CVF (50U/kg, i.v.) or with zymosan (10mg/kg, i.v.) along with ^{125}I-human serum albumin (HSA, 0,25μCi). After 30 mins ^{131}I-HSA (0.5μCi) was injected i.v. for 5 mins before sacrifice, as a marker of pulmonary intravascular volume. The activities of ^{125}I-HSA and ^{131}I-HSA in whole lung tissue were measured in a gamma counter and compared with that in plasma. Albumin accumulation was expressed as μl of plasma retained extravascularly in whole lung. The anti-mouse CD11b monoclonal antibody 5C6 was injected (1mg, i.v.) 15 mins before CVF or zymosan treatment. All lung tissue was processed for examination by light microscopy.

In control, saline-treated mice, accumulation of albumin in lung tissue after 30 mins was $0.7\pm0.1\mu l$ (mean±SEM, n=11). The albumin accumulation increased in the CVF-treated animals to $5.7\pm0.9\mu l$ (n=10, $p<0.01$) and to $6.7\pm1.7\mu l$ (n=7, $p<0.01$) in animals treated with zymosan. Pretreatment with 5C6 did not significantly alter the accumulation of albumin and the values determined were as follows: 5C6+CVF, $6.3\pm0.5\mu l$ (n=10); 5C6+zymosan, $7.8\pm1.7\mu l$ (n=6). Histological examination of the lung tissue revealed that there was extensive recruitment of neutrophils into the pulmonary microvessels in response to either CVF or zymosan treatment. In addition, neutrophils could be identified in small extracapillary vessels in CVF-treated mice but not in the animals treated with zymosan. There was no evidence for neutrophil migration into the alveolar spaces. Pretreatment with 5C6 did not alter the accumulation of neutrophils as seen by light microscopy.

Our results indicate that blockade of CD11b leukocyte adhesion molecule does not affect increased vascular permeability to albumin or neutrophil recruitment in response to complement activation by i.v. CVF or zymosan. This contrasts with our previous findings showing that 5C6 pretreatment reduced albumin accumulation in combined LPS and zymosan-treated mice (Miotla *et al*, 1993) and with studies of acute lung injury induced by CVF in the rat (Mulligan *et al*, 1993). We are currently investigating the mechanisms of CD11b-independent acute lung injury.

We would like to thank the National Asthma Campaign and the Ministry of Defence for support and Celltech for their generous gift of 5C6.

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249P PROTECTION FROM HISTAMINE-INDUCED BRONCHOCONSTRICTION BY PDE-INHIBITORS IN CONSCIOUS GUINEA-PIGS: EFFECT OF CHRONIC TREATMENT

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The phosphodiesterase (PDE) inhibitor, aminophylline, protects against the bronchoconstriction induced by antigen challenge in anaesthetised guinea-pigs, and this protection has been shown to persist after chronic treatment (Andersson & Bergstrand, 1981). This study follows the protection afforded by aminophylline and selective PDE inhibitors during chronic treatment of conscious animals.

Specific airway conductance (sGaw), using constant volume whole body plethysmography, was measured in conscious, sensitised male Dunkin-Hartley guinea-pigs (400-600g). Exposure to histamine (0.3mg/ml) via nebulised aerosol for 1 minute produced a bronchoconstriction which peaked at 2 min (84±1.8% reduction in sGaw). Aminophylline (30mg/kg s.c. in 50:50 DMSO:water for injection) given 30 minutes before a second histamine challenge, 24 hours later, protected against this bronchoconstriction (51±9.5% reduction in sGaw). Treatment with aminophylline (30mg/kg s.c. twice daily) was continued for 7 days. Further histamine challenges were made 30 minutes after the 1st daily injection on days 2 and 7. This resulted in a progressive reduction in the degree of protection with 62.7±7.2% and 75±2.3% reductions in sGaw, respectively (n=8).

In control experiments (DMSO:WF1 s.c. twice daily for 7 days) histamine induced a bronchoconstriction (30 mins after vehicle injection) on days 1 (80.6±2.4%), 2(80.2±1.5%) and 7 (65.5±9.2%) which were not significantly different from that before treatment (79.4±1.5%) (n=6). The bronchoconstriction was therefore reproducible over 7 days.

Similar experiments were carried out with the PDE inhibitors, isobutylmethylxanthine (IBMX, 1mg/kg, non-selective), SKF 95654 (3mg/kg, PDE III selective) and Ro 20-1724 (3mg/kg, PDE IV selective). Protection against the histamine-induced bronchoconstriction was demonstrated with all these drugs on the first day of treatment. The reduction in sGaw values were:- for IBMX; 86±2.1% (control) to 65.5±5.6% (day 1) (n=6), for SKF 95654; 80±2.1% (control) to 47.7±9.7% (day 1) (n=6) and for Ro 20-1724; 83±0.9% (control) to 59±3.1% (day 1) (n=6). After 7 days of treatment the degree of protection was not significantly different from that on day 1 with any of the drugs studied.

The adenosine P_1 receptor antagonist 8-phenyltheophylline (10mg/kg s.c. in DMSO:WF1, twice daily) did not show protection against histamine-induced bronchoconstriction on any day examined.

This study has shown that tolerance develops to the protective effect of aminophylline within 7 days, however no tolerance develops with specific PDE inhibitors.

Supported by SERC CASE award with SmithKline Beecham. We thank Dr. A.L. Rothaul for helpful advice.

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250P EFFECT OF Ba 679 BR, A NOVEL LONG-ACTING ANTICHOLINERGIC AGENT, ON CHOLINERGIC NEUROTRANSMISSION IN GUINEA-PIG AND HUMAN AIRWAYS

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Parasympathetic nerve-mediated bronchoconstriction is the major reversible component in chronic obstructive pulmonary disease, and contributes to nocturnal asthma through the diurnal variation of vagal tone. However, present anticholinergic drugs are sometimes inadequate in the treatment of these diseases because of their non-selectivity and short duration of action. We investigated the effect of Ba 679 BR (Ba), a novel antimuscarinic agent with a long duration of action (Disse *et al.*, 1993), on cholinergic neural responses in guinea-pig trachea and human bronchi *in vitro*.

Tissues were obtained from male Dunkin-Hartley guinea-pigs (300-500g; tracheal strips) and 7 donor and 2 recipient patients (age 12-59; 4 male) for heart/heart-lung transplantation. Isometric tension was recorded from these tissues by well defined methods. Electrical field stimulation (EFS; 4Hz, 0.5ms for 15s in every 4min) was applied to elicit cholinergic contractile responses and the effects of Ba, atropine and ipratropium bromide (IB) were studied. To evaluate M_2-receptor activity of these drugs we studied their effects on [^{3}H]-acetylcholine (ACh) release evoked by EFS from guinea-pig tracheal strips pretreated with [^{3}H]-choline (Wessler *et al.*, 1990).

Ba, atropine and IB inhibited EFS-induced contraction in a concentration-related manner with IC_{50} values of 0.17nM (Ba), 0.74nM (atropine) and 0.58nM (IB) in guinea-pig trachea. At concentrations that just evoked maximum inhibition of contraction, Ba (1nM) had a slower onset of action than atropine and IB (both 10nM); the times required to attain 50% of the maximum response [$T_{1/2}$(onset)]±s.e.mean were 34.8±3.5min, 3.8±0.5min and 7.6±1.2min, respectively (n=5). Furthermore, Ba had a markedly longer duration of action than atropine and IB. The time taken from wash-out of the test antagonists to 50% recovery of the contractile responses [$T_{1/2}$(offset)] were 540±34min, 31.6±3.7min and 81.2±23.5min, respectively. The slow onset and long duration of action of Ba (1nM) were also observed in human bronchi [IC_{50}=0.24nM, $T_{1/2}$(onset)=43.5±7.1min, $T_{1/2}$(offset)>300min, n=5]. Ba (1nM), as well as atropine and IB (both 10nM), facilitated [^{3}H]-ACh release evoked by EFS [%Enhancement of 43.0±9.0% (n=8, $p<0.05$), 34.2±14.5% (n=7, $p<0.05$) and 29.3±5.9% (n=6, $p<0.05$), respectively], suggesting that Ba also inhibits prejunctional muscarinic M_2-receptors. However, the facilitation of ACh release by Ba was lost 2h after wash out, when there was still complete blockade of cholinergic contractile responses evoked by EFS (an effect on airway smooth muscle M_3-receptor).

This data confirms binding studies which suggest that Ba may show "kinetic receptor subtype selectivity", M_3- over M_2-receptors (Disse *et al.*, 1993), and therefore it may offer improved efficacy over existing treatment. The very long duration of action of Ba in human bronchi suggests that Ba may be a useful drug to provide convenient therapy for patients with obstructive airway disease.

This work was supported by the Wellcome Trust, the National Asthma Campaign, the British Lung Foundation, Boehringer Ingelheim and Fujisawa/Fisons (Japan).

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251P SUPEROXIDE PRODUCTION BY NEUTROPHILS FROM NORMAL HORSES AND HORSES WITH CHRONIC OBSTRUCTIVE AIRWAY DISEASE

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Antigen-induced early neutrophil recruitment to the lungs has been observed in horses with chronic obstructive pulmonary disease (COPD), but not in normal horses (Fairbairn et al., 1993). Activation of circulating neutrophils has been demonstrated after antigen challenge of human asthmatic patients (Carroll et al., 1985). Before assessing the effects of antigen challenge on neutrophil activation in normal and COPD horses, the ability of equine peripheral blood neutrophils to generate superoxide in response to three stimuli has been evaluated.

Superoxide production by equine neutrophils was determined by measuring the superoxide dismutase-inhibitable reduction of cytochrome C (Sato et al., 1992) and the results expressed as nanomoles O_2^- per 10^6 cells after subtraction of background values. Phorbol myristate acetate (PMA) (0.005 - 500ngml^{-1}), serum treated zymosan (STZ) (0.05 - 1.58mgml^{-1}) and platelet activating factor (PAF) (10^{-6} - 10^{-5}M) in the presence and absence of cytochalasin B (5μgml^{-1}), were used as stimuli.

STZ and PMA caused similar dose related increases in O_2^- production by neutrophils isolated from normal and COPD horses. In contrast, PAF was found to be a weak stimulus, the maximum response to 10^{-5}M being 3.1 ± 0.3 nmolO_2^-/10^6 cells; n= 4 normal horses. STZ (1.58mgml^{-1}) and PMA (5ngml^{-1}) induced production of 22 ± 0.4 and 25.6 ± 0.9 nmolO_2^-/10^6 cells; n = 4 and 5 horses, respectively. The amount of superoxide produced by neutrophils from COPD horses in response to 10^{-5}M PAF was no different (2.2 ± 0.5 nmolO_2^-/10^6 cells; n=4). STZ (1.58mgml^{-1}) and PMA (5ngml^{-1}) caused production of 21.8 ± 0.2 and 22.4 ± 2nmolO_2^-/10^6 cells from 4 and 5 COPD horses, respectively. Prior incubation with cytochalasin B had little effect upon the PAF-induced response (3.4 ± 0.8 and 3.4 ± 0.5nmolO_2^-/10^6 cells; n=4 normal and COPD horses, respectively). Moreover, altering the concentration of cytochalasin B to 0.5 or 50μgml^{-1} did not enhance PAF-induced superoxide generation.

These results demonstrate that equine peripheral blood neutrophils, like those from other species, can produce superoxide radicals in response to soluble and particulate stimuli, although the response to PAF was small, and not apparently enhanced by cytochalasin B. No differences were observed between the responses of circulating cells from allergic horses in clinical remission and those from normal horses. Whether antigen challenge leads to changes in equine neutrophil superoxide generation is under investigation.

We are grateful to the Home of Rest for Horses for financial support. KAM is a Horserace Betting Levy Board Training Scholar

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252P EFFECTS OF ALVEOLAR MACROPHAGES ON ARACHIDONIC ACID (AA) METABOLISM IN RAT TRACHEAL EPITHELIAL CELLS IN PRIMARY CULTURE

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Different AA derived mediators appear to play an important role in the regulation of several airway functions and in the pathogenesis of obstructive airway diseases. The airway epithelium is known as a major source of AA metabolites. Therefore, the present experiments should characterize the metabolism of AA in isolated rat tracheal epithelial cells and test putative effects of alveolar macrophages.

Tracheae of newborn rats, cut into small pieces, were explanted onto culture dishes and cultured under conditions allowing selective proliferation of epithelial cells (Emura *et al.*, 1982). Confluent epithelial layers (after 4 weeks) were incubated with ^{3}H-AA for 16 hrs. After intensive washing, the cells were incubated for 3 consecutive 1 h periods in Krebs-HEPES medium and the outflow of ^{3}H-compounds was determined. ^{3}H-Compounds were separated by gradient reverse phase HPLC allowing the identification of PGE_2/D_2, TxB_2, LTB_4, 15-, 12- and 5-HETE and AA.

In medium of unstimulated epithelial cells, the fraction coeluting with ^{3}H-PGE_2/D_2 represented the main fraction (2130 ± 339 DPM, mean ± s.e.m., n=25) compared to ^{3}H-LTB_4 (250 ± 42 DPM) and ^{3}H-AA (450 ± 52 DPM) or other metabolites (< 150 DPM). Incubation with the calcium ionophore A 23187 (10 μM) caused a 2.7fold increase in the outflow of ^{3}H-PGE_2/D_2 without major changes in outflow of ^{3}H-AA and ^{3}H-LTB_4. Incubation in the presence of phorbol 12-myristate 13-acetate (PMA, 100 nM) did not cause an increase in the outflow of any ^{3}H-AA metabolite.

Alveolar macrophages (freshly prepared by broncho-alveolar lavage of isolated rat lungs) were also labelled with ^{3}H-AA. They showed only a very low rate of spontaneous outflow of ^{3}H-AA and ^{3}H-AA-metabolites. PMA did not cause major changes in the outflow of ^{3}H-LTB_4 and ^{3}H-AA, but selectively increased (about 6fold) the outflow of ^{3}H-TxB_2 and ^{3}H-prostaglandins. On the other hand, A 23187 (10 μM) caused a large increase in ^{3}H-LTB_4 outflow (80fold) compared to an only 6fold increase in ^{3}H-TxB_2. Addition of unlabelled alveolar macrophages to epithelial cells, which had been labelled with ^{3}H-AA, caused a marked rise in release of ^{3}H-AA (40fold) and of several lipophilic metabolites (about 20fold) and a 5fold increase in ^{3}H-prostanoid release, whereas alveolar macrophages-conditioned medium caused only a substantial liberation of ^{3}H-AA (20fold increase) without significant effects on the outflow of ^{3}H-prostanoids or other ^{3}H-metabolites.

In conclusion, AA metabolism in alveolar macrophages and tracheal epithelial cells is differentially regulated via protein kinase C and free intracellular calcium. Addition of alveolar macrophages to epithelial cells causes liberation of AA, and this is accompanied by an enhanced formation of different metabolites. Humoral factors as well as cell to cell contacts appear to be involved in the described effects of alveolar macrophages. The described co-culture system may be an useful model to study basic mechanisms involved in inflammatory airway diseases.

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253P EFFECT OF THE PHOSPHODIESTERASE (PDE) III/IV INHIBITOR, ZARDAVERINE, ADMINISTERED ACUTELY AND CHRONICALLY ON PULMONARY CELL INFLUX IN OVALBUMIN SENSITISED GUINEA-PIGS

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Recently it has been suggested that theophylline may possess anti-inflammatory activity (Ward *et al.*, 1993). However the mechanism underlying this phenomenon is poorly understood. It has been shown that a single high dose of theophylline can inhibit eosinophil infiltration into the airways of immunized guinea pigs (Gristwood *et al.*, 1991) but the doses used are much higher than those used clinically. Type III and IV PDE isozymes have been characterised in a variety of inflammatory cells and to test whether type III and type IV PDE isozymes represent a potential drug target in airway allergen-induced cell accumulation, the specific III/IV PDE inhibitor, zardaverine was examined.

The present study has compared the effect of a single dose of zardaverine (30mgkg^{-1} i.p.) 1 h prior to antigen, ovalbumin (OA) (100µgml^{-1} for 1h) challenge with that of twice daily dosing of zardaverine for 7 days (3mgkg^{-1} i.p.) and subsequently 1h prior to OA exposure (100µgml^{-1} for 1h) on pulmonary inflammatory cell influx 18-24 h later in OA-sensitized guinea pigs, according to the method described by Seeds *et al.*, (1991). Data is expressed as mean ± s.e.mean and cells are expressed as 10^4 cellsml^{-1}. Groups were compared with time-matched controls by Dunnett's test. *P<0.05 versus allergen.

Table 1

	Sham/Vehicle		Allergen/Vehicle		Allergen/Zardaverine	
	Acute	*Chronic*	*Acute*	*Chronic*	*Acute*	*Chronic*
	n=7	n=7	n=7	n=8	n=9	n=12
Total cells	29.5±3.2	42.3±4.9	34.0±2.5	36.8±4.2	41.2±6.1	*23.7±3.0
Neutrophils	1.3±0.6	1.3±0.3	1.7±0.5	0.7±0.3	6.6±5.1	2.1±0.7
Eosinophils	4.1±0.9	4.3±1.4	13.0±3.1	11.3±1.7	12.5±3.3	*4.1±1.4
Mononuclear cells	24.1±3.5	36.7±3.5	19.3±1.8	24.8±4.1	22.1±2.3	17.5±3.2

Exposure of allergen immunized animals to aerosolised antigen produced a significant increase in eosinophil infiltration compared with sham treated animals, without altering the total cell number. Zardaverine had no significant effect on neutrophil or mononuclear cell numbers compared with vehicle treated animals. Antigen-induced eosinophil infiltration was significantly inhibited by low dose chronic treatment for 1 week with zardaverine whereas administration of a single high dose of zardaverine 1 h prior to antigen challenge had no significant effect on eosinophil infiltration. These results suggest that a period of chronic treatment with zardaverine is necessary to cause inhibition of eosinophil accumulation.

K.H.B is supported by the M.R.C.

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254P THE EFFECT OF RICIN ON IgE PRODUCTION, ANTIGEN-INDUCED AIRWAY INFLAMMATION AND AIRWAY HYPERREACTIVITY IN THE BROWN NORWAY RAT

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Human allergic asthma is characterized by airway eosinophilia and airway hyperreactivity (AHR) and is often associated with increased production of antigen-specific IgE (Pride, 1992). Ricin augments IgE production in rats by a mechanism thought to involve depletion of suppressor CD8$^+$ T lymphocytes allowing increased production of IL-4 by helper CD4$^+$ T lymphocytes (Diaz-Sanchez *et al.*, 1993). We have investigated the possible link between IgE titre and airway inflammation and AHR induced by antigen challenge in this species.

Male, Brown Norway rats were sensitized (day 0) and boosted (days 12 and 21) with ovalbumin (100 µg, i.p.) with or without ricin (50 ng day 0, 10 ng days 12 and 21, i.p.). Serum titres of antigen-specific IgE on day 30 (time of peak response) were determined by radioallergosorbent assay. Rats were challenged (days 30, 32 and 34) with inhaled ovalbumin (nebulized 10 mg/ml solution, 20 min). 24 hours after the last challenge, airway reactivity was determined in anaesthetized (sodium pentobarbitone, 60 mg/kg, i.p.) animals as the dose of acetylcholine (inhaled aerosol, 8 sec) required to increase pulmonary resistance by 150 cmH_2O/(l/sec) (PD_{150}). Bronchoalveolar lavage (BAL) was then performed and total and differential cell counts made. Statistical significance was determined using Dunnett's multiple comparison test.

Antigen-specific IgE titre was significantly increased from 481 ± 21 U / ml to 1852 ± 43 U / ml in sensitized rats and was significantly increased still further to 19658 ± 969 U / ml in animals which also received ricin. Antigen challenge caused BAL eosinophilia and neutrophilia together with AHR. Eosinophilia and neutrophilia, but not AHR were significantly increased in rats treated with ricin (Table 1).

Table 1. The effect of ricin on antigen-induced airway inflammation and AHR in the sensitized Brown Norway rat.

Group	n	Treatment		Eosinophils (x10^3/ml)	Neutrophils (x10^3/ml)	Acetylcholine PD_{150} (nM)
1	11	Unsensitized,	unchallenged	7 ± 3	7 ± 3	64 ± 8
2	4	Ovalbumin + ricin,	unchallenged	9 ± 4	3 ± 1	60 ± 5
3	9	Ovalbumin,	challenged	57 ± 17 *	51 ± 19 *	39 ± 7 *
4	9	Ovalbumin + ricin,	challenged	166 ± 37 * †	160 ± 67 * †	34 ± 6 *

*** $p < 0.05$ compared with group 1. † $p < 0.05$ compared with group 3.**

Ricin produced a 10-fold increase in antigen-specific IgE and augmented antigen-induced eosinophilia and neutrophilia in BAL fluid. However, the increased inflammation was not accompanied by a further increase in AHR. These data demonstrate that AHR reaches a plateau and that the number of inflammatory cells and serum IgE levels are not causally related to AHR.

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255P FUNCTIONAL MODULATION OF RECOMBINANT $GABA_A$ RECEPTORS BY ACTIVATION OF PROTEIN KINASE C

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Phosphorylation of ligand-gated ion channels is a mechanism whereby covalent modification of the receptor structure may lead to a long-term modulation of receptor function (Swope *et al.*, 1992). A number of consensus sequences for phosphorylation by protein kinase C on γ-aminobutyric acid ($GABA_A$) receptors have been identified involving serine residues located on the putative large intracellular loops between transmembrane domains M3 and M4 of β1, γ2 short (γ2S) and γ2 long (γ2L) $GABA_A$ receptor subunits (Moss *et al.*, 1992). This study addresses whether phosphorylation affects the function of recombinant $GABA_A$ receptors. Mouse cDNAs encoding wild-type α1, β1, γ2S and γ2L $GABA_A$ receptor subunits, or mutant cDNAs, where the serine (S) residues have been replaced with an alanine (A) to form β1(S409A), γ2S(S327A) and γ2L(S327A,S343A), were co-expressed in *Xenopus laevis* oocytes and embryonic human kidney cells (A293). Expressed receptors were studied using biochemical and intracellular and patch clamp electrophysiology.

Phosphorylation of the β1 subunit by phorbol 12-myristate 13-acetate (PMA) was assessed using ^{32}P and subunit specific antisera. PMA increased the level of phosphorylation on the β1 subunit which could be localised to a single serine residue at position 409. Mutation of the serine at position 409 to alanine abolished the increase in phosphorylation. Membrane currents induced by GABA (I_{GABA}, 0.1-500 μM) on oocytes (Kellenberger *et al.*, 1992) and kidney cells expressing $GABA_A$ receptors were inhibited after incubation with 250-500 nM PMA. The inactive 4α-phorbol 12,13-didecanoate (500 nM) failed to antagonise I_{GABA}. PMA differentially reduced I_{GABA} in the following order according to the $GABA_A$ receptor subunit composition: α1β1γ2L ≥ α1β1γ2S >> α1β1. The mechanism of inhibition by PMA determined from equilibrium concentration-response curve analyses is of the mixed/non-competitive type. The EC_{50}s were determined for GABA on the following recombinant receptors and showed slight variation in the presence of PMA (α1β1: mean ± s.e. mean, 5.8 ± 0.6μM (control), 4.3 ± 0.2 (+ 250nM PMA); α1β1γ2S: 10 ± 0.2 (control), 13 ± 0.4 (+PMA); α1β1γ2L: 10.2 ± 0.5μM (control), 9.4 ± 0.5μM (+PMA), n = 18). Current-voltage relationships revealed that the antagonism by PMA was essentially voltage-independent and the reversal potential for GABA was unaffected (-20 mV (control); -23mV (+PMA) for α1β1γ2S) Co-expression of α1 with the mutant forms of β1, γ2S or γ2L removed the modulation of I_{GABA} by PMA. These results suggest that activation of PKC using phorbol esters can negatively modulate $GABA_A$ receptor function which is dependent on receptor subunit composition and possibly the number of phosphorylation sites.

This work is supported by the MRC and Wellcome Trust.

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256P IDENTIFICATION OF A SINGLE AMINO ACID ON THE β SUBUNIT OF THE $GABA_A$ RECEPTOR REQUIRED FOR THE MODULATORY ACTIONOF LORECLEZOLE

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Loreclezole ((z)-1-[2-chloro-2-(2,4-dichlorophenyl)ethenyl]-1,2,4-triazole) is a broad spectrum anticonvulsant effective in a number of seizure models in a variety of species. The anticonvulsant effect of loreclezole can be inhibited by the benzodiazepine inverse agonists β-CCE and CGS 8216 but not by the antagonist Ro15-1788 (Vaught and Wauquier, 1991), suggesting that it does not act directly at the benzodiazepine binding site but at a novel modulatory site. Expression of subunit combinations in Xenopus *oocytes* has now allowed characterization of the interaction of loreclezole with the $GABA_A$ receptor. Wafford *et al.* (submitted) have demonstrated that the modulatory site for loreclezole is found on the β subunit and furthermore is highly selective for receptors containing the β_2 or β_3 subunit over those containing β_1.

We report here the results of a study undertaken to identify the determinants required for the modulatory effect of loreclezole. Initially chimeric β_1/β_2 subunits were constructed and co-expressed with α1 and γ2 in *Xenopus* oocytes. It was found that the region of the β_2 subunit encompassing the putative transmembrane spans TM1 to TM3, when introduced into the corresponding region of the β_1 subunit, was sufficient to confer potentiation of GABA-evoked currents by loreclezole. Alignment of the three β TM1-TM3 peptide sequences reveals four positions which have identical residues in β_2 and β_3 subunits but not in β_1 (positions 254, 289, 307 and 324 of the human β_2 subunit, Hadingham *et al.*, in press). The fourth position is within the putative second intracellular loop and was discounted. The β_1 subunit was mutated to the equivalent β_2/β_3 sequences at each of the three remaining positions by site-directed mutagenesis. Mutation of Ser 290 of β_1 (equivalent position 289 of β_2) to Asp resulted in potentiation by loreclezole to about 60% of control (β_2) levels. No such response was observed from the other β_1 point mutants. To confirm this observation two further mutations were made; β_2 Asn 289 to Ser and β_3 Asn 290 to Ser. In both cases, potentiation by loreclezole was lost, confirming the importance of this residue. From these data we conclude that Asn 289 of β_2 and Asn 290 of β_3, which are located at the carboxy terminal end of the TM2 domain, are required for the modulatory effect of loreclezole on GABA-A receptors. Whether this Asn residue is required for the binding of loreclezole, or for the conformational change which leads to the modulatory actions, is currently unknown.

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257P THE FUNCTIONAL PROPERTIES OF A *DROSOPHILA* GABA SUBUNIT EXPRESSED IN *XENOPUS* OOCYTES

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GABA plays a fundamental role in mediating neuronal inhibition in the invertebrate nervous system. However, relatively little is known of the receptors which mediate its' actions. Recently, a cDNA, isolated from embryonic *Drosophila* has been identified which encodes a functional GABA-gated chloride channel when expressed in *Xenopus laevis* oocytes (ffrench-Constant *et al.*, 1993). We have isolated a related cDNA from an adult *Drosophila melanogaster* head library and we report here on the functional properties of this clone, when expressed in *Xenopus laevis* oocytes. Quantitative data are reported as the mean ± s.e.m.

At a holding potential of -60mV, all oocytes (n = 115) injected 2-12 days previously with cRNA encoding the *Drosophila* receptor subunit responded to 3mM GABA with an inward current of 267 ± 72nA (n = 25). Analysis of GABA and muscimol concentration-response curves revealed EC_{50} values of 152 ± 10μM and 196 ± 22μM and Hill coefficients of 1.72 ± 0.07 and 1.8 ± 0.05 (all n = 5) respectively. Glycine, glutamate and baclofen (all at 1mM, n = 4) were inactive in this respect. A linear relationship between the GABA reversal potential (E_{GABA}) and the log of the extracellular Cl^- concentration ($[Cl^-]_o$) was observed, a decade reduction in $[Cl]_o$ changing E_{GABA} by 53mV. Hence, the GABA-induced current is mediated by chloride ions.

GABA (150μM)-induced currents were blocked by picrotoxin (IC_{50} = 46 ± 6nM, n = 4), TBPS (IC_{50} = 2.2 ± 0.2μM, n = 4) and La^{3+} (IC_{50} = 12.2 ± 1.6μM, n = 4) but were unaffected by bicuculline, strychnine, and Zn^{2+} (all 100μM, n = 4). GABA (50μM)-induced currents were enhanced by pentobarbitone (30μM - 1mM; max effect = 554 ± 113% control, n = 5) but the neurosteroid 5α-pregnan-3α-ol-20-one produced only a modest effect (max. = 206 ± 16% of control, n = 5) at the relatively high concentration of 10μM. Flunitrazepam (3μM) was inactive (n = 4).

The *Drosophila* cDNA encodes a GABA-activated chloride channel which exhibits a novel pharmacology in comparison to mammalian GABA receptors. As this subunit functions efficiently as a homomer, it should be ideal for mutagenesis studies designed to better define drug binding sites on the GABA receptor channel complex.

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258P STRUCTURE AND PHARMACOLOGICAL CHARACTERISATION OF δ-CONTAINING $GABA_A$ RECEPTORS IN RAT BRAIN

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The cytoplasmic loop region of the δ-subunit of the $GABA_A$ receptor was amplified using the polymerase chain reaction and engineered into the expression vector pRSET5a. The polypeptide was expressed in *E.Coli*, purified by preparative SDS/PAGE and used to raise polyclonal antisera in rabbits. These specifically identified the expressed fragment by Western blot but did not cross react with analogous polypeptides from the γ1,γ2 or γ3-subunits. The δ-antiserum (30μl) maximally immunoprecipitated 3-4pmol of [^{3}H]muscimol binding sites giving a titre of 0.1×10^{-6} mol of [^{3}H]muscimol binding sites per litre of serum. It also detected the entire δ-subunit in rat brain membranes by Western blot, identifying a polypeptide of 55KDa. Receptors containing a δ-subunit accounted for 10.7± 2% (n=4) of $GABA_A$ receptors solubilised from whole rat brain membranes as deduced from quantitative immunoprecipitation experiments, the largest population being in the cerebellum where 27.6 ± 5.3% (n=3) of $GABA_A$ receptors contained a δ-subunit. Polyclonal antisera immunoprecipitated $GABA_A$ receptors from other brain regions consistent with the reported distribution of δ-subunit mRNA (Wisden *et al.*, 1992, Laurie *et al.*, 1992). Receptors immunoprecipitated with the δ-specific antisera exhibited an atypical profile with respect to their radioligand binding properties. They were labelled by [^{3}H]muscimol, but did not bind benzodiazepine site ligands with high affinity. The pharmacology of the GABA binding site on receptors immunoprecipitated from cerebellum with the δ-antiserum was compared with those immunoprecipitated with the γ2-antiserum. The rank order of potency of a series of 6 compounds to compete for [^{3}H]muscimol binding sites was similar in these two populations, but muscimol had a significantly higher affinity for receptors containing the δ-subunit (0.49 + 0.07nM for δ versus 2.96 + 1.1nM for γ2, n=4). These receptors therefore comprise a novel population of $GABA_A$ receptors which do not bind benzodiazepines and have a 6-fold higher affinity for muscimol. Coexistence of the δ-subunit with γ or α-subunits was investigated by purifying receptor on a variety of immunoaffinity resins, separating the subunit components by SDS/PAGE transferring to nitrocellulose and probing with δ-subunit specific antibodies. The δ-subunit was found predominantly to coexist in the same receptor complex as the α_6 subunit. Very small populations of $\alpha_5\delta$ and $\alpha_3\delta$ may also be present. The δ-subunit could not be detected in combination with any γ-subunit nor in combination with the α_1 or α_2 subunit.

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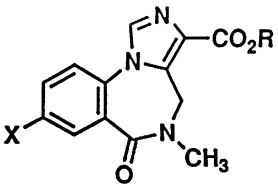
259P SUBSTITUTION AT POSITION 8 OF IMIDAZO-BENZODIAZEPINE-6-ONES IS A DETERMINANT FOR $GABA_A$ α5-SUBTYPE ACTIVITY

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The GABA-A receptor family comprises multiple subtypes of receptor which have an agonist binding site and a modulatory site with high affinity for benzodiazepines. The benzodiazepine site is believed to be formed, at least in part, by the α-subunit of the GABA-A receptor since compounds have different affinities depending on the type of α–subunit present. To date, most discriminatory compounds exhibit the highest affinity for receptors containing the α1-subunit, and some, such as zolpidem have very low affinity for α5-containing receptors (Pritchett *at al.*, 1990, McKernan *et al.*, 1991). In order to investigate the structural requirements for selectivity at the benzodiazepine site a series of 8-substituted imidazobenzodiazepine-6-ones have been tested at several subtypes of the GABA-A receptor. The ability to displace [^{3}H]Ro15-1788 from cells expressing human GABA-A receptors of structure α1β3γ2, α2β3γ2 and α5β3γ2 (Hadingham *et al.*, 1993) was tested. Ki values (nM) were obtained as shown in Table 1.

Table 1

X	R	α1β3γ2	α2β3γ2	α5β3γ2
NCS	t-butyl ester	17.1 ± 3	20.8 ± 4	2.4 ± 0.4
Cl	t-butyl ester	14.8 ± 1.8	23.0 ± 3.9	0.66 ± 0.05
NO_2	t-butyl ester	13.4 ± 2.5	58.8 ± 8.4	3.4 ± 0.7
I	t-butyl ester	9.6 ± 1.9	16.7 ± 3.6	0.32 ± 0.05
Br	t-butyl ester	11.4 ± 3.5	13.0 ± 2.7	0.37 ± 0.18
N_3	ethyl ester (Ro15-4513)	8.8 ± 0.9	8.1 ± 2.4	1.0 ± 0.23
F	ethyl ester (Ro15-1788)	0.92 ± 0.04	1.05 ± 0.05	0.45 ± 0.04



It is concluded that varying the substituent at the 8-position is an important determinant of α5-selectivity. Further the presence of Br at this position yields a compound with 30-fold selectivity for α5-containing receptors over α1. This is the most α5-selective compound reported to date.

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260P THE EFFECTS OF DIFFERENTIATION OF IMR32 CELLS WITH PGE_1 ON $GABA_A$ RECEPTOR DENSITY

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We have reported previously that the IMR32 human neuroblastoma cell line possesses a functional $GABA_A$ receptor not associated with specific benzodiazepine binding sites (Anderson *et al*, 1993). In this study we have examined the effects of a range of potential differentiating agents on [^{35}S]-TBPS binding and [^{3}H]-flunitrazepam binding to the GABA receptor of IMR32 cells.

IMR32 cells were maintained in Eagle's MEM supplemented with: 10% heat-inactivated foetal calf serum, 1% L-glutamine, 1% non-essential amino acids, and 50 μgml^{-1} gentamicin. Cells were grown in differentiating agents for 8-11 days at the following final concentrations: retinoic acid (0.03 μgml^{-1}), dibutyryl cAMP (0.5 $mgml^{-1}$), prostaglandin E_1 (10 μgml^{-1}), NGF (0.2 μgml^{-1}) phorbol myristate acetate (10 $ngml^{-1}$) and papaverine (10 μgml^{-1}). [^{35}S]-TBPS binding and [^{3}H]-flunitrazepam binding were performed as described by Cross *et al* (1989).

NGF, PGE_1 and dibutyryl cAMP stimulated neurite outgrowth in IMR32 cells, however none of these agents induced significant [^{3}H]-flunitrazepam binding. PGE_1-treated cells showed a significant increase in [^{35}S]-TBPS binding compared with controls, which was characterised by an increased B_{max} with no change in K_d (Table 1).

Table 1 [^{35}S]-TBPS binding to rat cortex and IMR32 cell membranes

	Rat cortex	IMR32 (control)	IMR32 (PGE_1-treated)
Kd (nM)	99.1 ± 3.8	60.9 ± 15.8	50.6 ± 18.5
Bmax (fmol/mgprotein)	4324 ± 640	281 ± 68	593 ± 120*

Values are mean ± s.e.mean (n=4) *$p<0.02$ vs control.
Displacement of [^{35}S]-TBPS binding by the $GABA_A$ agonists GABA and muscimol was identical in control and PGE_1-treated cells. We conclude that differentation of IMR32 cells by PGE_1 does not change the properties of the $GABA_A$ receptor although the number of receptors is increased.

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261P THE EFFECTS OF 5-AMINOLAEVULINIC ACID AND γ-AMINOBUTYRIC ACID ON [^{3}H]-FLUNITRAZEPAM BINDING TO RAT CEREBRAL CORTEX MEMBRANES

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The central and peripheral neurological symptoms of acute intermittent porphyria (AIP) may be mediated by the haem precursor 5-aminolaevulinic acid (ALA) which accumulates as a result of an enzymatic defect in the haem biosynthetic pathway. ALA exerts pharmacological effects on some *in vitro* preparations possibly via an interaction at the receptor complex for the inhibitory neurotransmitter, γ-aminobutyric acid (GABA) (Cutler et al. 1991). ALA and GABA are structural analogues and ALA may produce a neurological effect, in AIP, by competing with GABA for receptor sites. Benzodiazepines (BDZ) bind to the $GABA_A$ receptor complex and both BDZ and GABA mutually potentiate the binding of the other to the receptor complex (Heusner and Bosmann,1981). The present experiments examined the effects of both GABA and ALA on the binding of [^{3}H]-flunitrazepam to the $GABA_A$ receptor complex of rat cerebral cortex membranes.

Membranes were prepared by differential centrifugation of homogenized cerebral cortices from male Wistar rats. Binding assays were carried out in 50mM Tris buffer at 0°C for 30min. The binding of six concentrations of [^{3}H]-flunitrazepam (0.45-15nM) were examined in buffer alone or in the presence of either GABA (10μM and 100μM) or ALA (10μM and 100μM). Cold clonazepam (5μM) was used to determine non-specific binding. Scatchard analysis was carried out to obtain Bmax and Kd values.

Table 1

	Kd (nmoles)	**BMax**(fmoles/mgprotein)		**Kd** (nmoles)	**BMax**(fmoles/mg)
Control (n=7)	1.47±0.17	2022±114	Control (n=5)	1.59±0.26	2017±172
ALA 10μM (n=7)	1.39±0.13	2117±59	ALA 100μM(n=5)	1.28±0.03	1993±171
GABA 10μM (n=7)	*1.15±0.14	1917±62	GABA 100μM (n=5)	*1.04±0.03	2046±190

Table 1 shows that GABA at both 10 and 100μM caused a significant decrease in the Kd of the ligand (*$p<0.05$) whereas the number of receptor sites (Bmax) remained unaltered. ALA had no effect on Kd values.Bmax values were also unalterd.

The results confirm that GABA enhances BDZ binding to rat cerebral cortex membranes. In this study, any effects of ALA on BDZ binding were too small to reach statistical significance.

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262P GABAERGIC MODIFICATION OF 4-AMINOPYRIDINE-INDUCED EPILEPTIFORM ACTIVITY IN THE RAT ENTORHINAL CORTEX

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We have recently described the effects of the convulsant agent, 4-aminopyridine (4-AP) on slices of entorhinal cortex (EC) and hippocampus (Jones and Lambert, 1992). In brief, 4-AP induces ictal-like tonic-clonic electrographic seizures in the EC which last for many seconds and recur every few minutes, while concurrently in the adjacent CA3 area much briefer (<500 ms) high frequency (1-3 Hz) synchronous discharges are recorded. Recently it was reported that blockade of $GABA_A$-inhibition with bicuculline converts the tonic-clonic discharges of the EC into persistent tonic activity which essentially resembles a persistent, status epilepticus-like state (Hunter et al, 1993). We have also been looking at manipulations of GABA-inhibition on 4-AP induced activity and report some of these results here. Experiments were conducted in combined slices of rat EC, temporal cortex and hippocampus maintained *in vitro* at the interface between artificial CSF (at 34°C) and carbogen gas (95% O_2, 5% CO_2). Extracellular recordings were made with tungsten electrodes.

In 8 slices, perfusion with 4-AP (100 μM) induced long lasting (20-65 s, mean ± S.D. 28±13 sec) discharges in the EC at intervals of 75-440 s. Perfusion with the $GABA_A$ antagonist, bicuculline (5 μM, n=5) resulted in conversion of long lasting discharges occurring at intervals of 188±57s to rapidly recurring persistent discharges (9.9±1.3 event per min) as reported previously (Hunter et al, 1993). In 2 other slices which initially did not exhibit the long lasting tonic-clonic events, these appeared briefly in bicuculline (5 μM) before being rapidly converted to recurrent, persistent activity. Addition of the $GABA_B$-antagonist CGP 55845 (0.5-1.0 μM) increased the frequency of long lasting discharges, shortening the interval between them from 174±28s to 111±24s (n=4). Neither the amplitude nor form of the discharges were detectably altered. In three further slices, long lasting discharges were not seen in the control situation but appeared after the addition of CGP 55845, occurring at intervals of 132±34s. The recurrent persistent discharges seen with bicuculline were never observed with CGP 55845 alone. The GABA-uptake inhibitor, tiagabine (20 μM) abolished the long lasting discharges in the EC and this applied whether the discharges occurred with 4-AP alone (n=3) or whether they were initiated by the addition of CGP 55845 (n=2). However, if recurrent persistent discharges were induced by bicuculline then tiagabine only slowed the frequency of this activity (10.5±1.4 to 7.3± 1.9 events per min, n=3). Finally, the benzodiazepine, midazolam (2.0 μM), which might be expected to potentiate $GABA_A$-inhibition, also abolished the long lasting discharges in EC (n=4) and reduced the frequency and amplitude of recurrent persistent activity recorded in 4-AP plus bicuculline (n=3).

Thus, it is clear that both $GABA_A$ and $GABA_B$ receptors are involved in the control of epileptiform discharges induced by 4-AP in the EC although the Cl^--dependent inhibition probably plays a greater role. The ability of a GABA-uptake blocker to prevent the occurrence of ictal-like discharges suggests this could prove to be a fruitful approach to anticonvulsant therapy.

I thank the Wellcome Trust for financial support, Ciba-Geigy for the gift of CGP55845 and Novo-Nordisk for tiagabine

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263P BACLOFEN MODULATION OF VOLTAGE-GATED Ca^{2+} CHANNELS IN RAT CULTURED CEREBROCORTICAL NEURONES

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The function of voltage-gated Ca^{2+} channels in mammalian dorsal root ganglion (DRG) cells can be modulated by the neurotransmitter GABA acting at $GABA_B$ receptors (Dunlap 1981; Desarmenien *et al.* 1984, Scott & Dolphin 1990). GABA receptor activation can also induce a postsynaptic hyperpolarisation in higher centre neurones e.g. hippocampal pyramidal cells, via an increase in K^+ conductance (Newberry and Nicoll 1984). Since the significance of Ca^{++} in the responses to $GABA_B$ receptor activation in cultured cerebrocortical neurones has not yet been explored, we have examined and characterised the action of the $GABA_B$ agonist, (-)baclofen on internal calcium concentration ($[Ca^{2+}]_i$) and Ca^{2+} conductance in these cells.

Tight-seal whole-cell recordings and $[Ca^{2+}]_i$ measurements were performed on embryonic cerebral cortex neurones from rat primary culture (E18). Cortical neurones were loaded with 2μM Fura/2 AM at 37°C for 30 min in Krebs' solution. Depolarisation by 35mM KCl induced a reversible increase in $[Ca^{2+}]_i$ monitored by a microspectrofluorimetric technique. For whole-cell recording, single cells were voltage-clamped at -80mV and stepped to 0mV. The patch pipette solution for calcium current (I_{Ca}) isolation was as follows (mM) TEA-Cl 120, $MgCl_2$ 1, Na_2ATP 4, HEPES 5, BAPTA 10 and $CaCl_2$ 100nM, pH 7.2. The external HEPES solution consisted of (in mM) TEA-Cl 120, $CaCl_2$ 2.5, $MgCl_2$ 1, HEPES 10, Glucose 10 and 1μM tetrodoxin (TTX), pH adjusted to 7.2. All experiments were performed at room temperature.

Under voltage-clamp, depolarisation evoked a slowly inactivating inward current. This current was identified as a I_{Ca} because it was abolished in Ca^{2+} free solution containing 5mM EGTA (n=5) and was increased when Ba^{2+} replaced Ca^{2+} (n=5). Elevation of $[Ca^{2+}]_i$ by 35mM KCl was also abolished in Ca^{2+} free solution (n=5). Baclofen reversibly reduced both depolarisation-induced Ca^{2+} elevation and I_{Ca} to 30-35% maximum in each case. The inhibition of $[Ca^{2+}]_i$ elevation by baclofen was concentration dependent (0.1-50μM) with an IC_{50} value of 0.78μM. This value is similar to that reported by Tatebayashi & Ogata (1992) in cultured rat DRG neurones. Further experiments were carried out using the $GABA_B$ receptor antagonist CGP 35348 (p-(3-aminopropyl)-p-diethoxymethyl phosphinic acid). This compound reversibly reduced the inhibitory action of 10μM baclofen by 82 ± 6.1% (mean ± s.e. mean, n=6). Pre-incubation of cells with either baclofen or CGP 35348 in low KCl did not induce any detectable change in $[Ca^{2+}]_i$ or I_{Ca}.

In summary, (-)baclofen can modulate voltage-dependent Ca^{2+} channels and $[Ca^{2+}]_i$ in cultured cerebrocortical neurones. The reversible blocking action of CGP 35348 supports the view that (-)baclofen is acting via $GABA_B$ receptors in these cells.

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264P BACLOFEN INHIBITS WATER INTAKE INDUCED BY SYSTEMIC ADMINISTRATION OF THE ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITOR, CAPTOPRIL

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Systemic administration of low doses of captopril increases water intake in rats by elevating angiotensin II levels in the brain (Grossman, 1992). We have previously shown that s.c. administration of baclofen inhibits water intake in 16h water-deprived rats (Ebenezer et al, 1992), and preliminary experiments in our laboratory have also indicated that baclofen can inhibit drinking induced by central administration of angiotensin II. The present experiments were therefore conducted to investigate the effects of baclofen on captopril-induced drinking.

Male Wistar rats (body weight 390-440g) that were not water-deprived (n=8) or deprived of water for 5h (n=8), were injected s.c. with either saline followed by saline, saline followed by baclofen (2.0 mg kg^{-1}), captopril (2.5 mg kg^{-1}) followed by saline or captopril (2.5 mg kg^{-1}) followed by baclofen (2.0 mg kg^{-1}). Fifteen minutes separated the two injections. A repeated measures design was used with each of the rats receiving all of the treatments. Water intake was measured over a 2h period as previously described (Ebenezer et al, 1992). The results of these experiments are shown in Table 1.

Table 1. The effect of baclofen on captopril (2.5 mg kg^{-1}) induced drinking in rats.

TREATMENT	Non-Deprived	Water-Deprived
Saline-Saline	0.3 ± 0.06	0.5 ± 0.13
Saline-Baclofen	0.1 ± 0.03	0.6 ± 0.11
Captopril-Saline	1.4 ± 0.16*	2.1 ± 0.30*
Captopril-Baclofen.	0.6 ± 0.10	0.4 ± 0.13

All results are expressed as mean water intake (mls per 100g body weight) ± s.e.mean. *P<0.005 analysed by Dunnett's t-distribution.

The results of this study show that baclofen inhibits captopril induced drinking in non-deprived and mildly water-deprived rats. It is now well established that systemic administration of captopril increases central levels of angiotensin II, and it is possible that it is this effect that is responsible for the increase in water consumption (Grossman, 1992). The present observations are consistent with the notion that systemically administered baclofen acts centrally to inhibit the effects of angiotensin II on water intake.

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265P INVESTIGATIONS ON THE UPTAKE AND RELEASE OF [^{3}H]GABA FROM PERINATAL HYPOTHALAMUS IN PRIMARY CELL CULTURE

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It is generally accepted that the inhibitory amino acid, gamma-aminobutyric acid (GABA), plays an important role in regulating neuroendocrine function by modulating the release of hypothalamic neuropeptides involved in the control of anterior pituitary function. However, little is known concerning the mechanisms regulating the hypothalamic GABAergic neurones which may be implicated in processes underlying steroid hormone feedback and the ontogeny of the neuroendocrine system. Therefore using primary cultures of foetal rat hypothalamic tissue maintained in defined medium, we have investigated the functional activity of hypothalamic GABA - ergic neurones, using tritiated GABA ([^{3}H]GABA) to study the uptake and release mechanisms. Cells were incubated with [^{3}H]GABA at a concentration of 0.1µCi/well of cells, (2.5nmol, specific activity - 91Ci/mmol) at 37°C for 10 min. Uptake was terminated by washing the cells in ice-cold Earles Balanced Salt Solution (EBSS) and excess label removed by extensive rinsing with the same medium. Uptake and release were measured respectively by liquid scintillation spectrometry after solubilization of the cells or collection of the incubation medium after a specified time period.

The uptake of [^{3}H]GABA (expressed as d.p.m./well of cells, 5.6 x 10^5 cells/well), was concentration, time and temperature dependent. Specific uptake, (i.e. uptake at 37°C minus that at 4°C), increased dramatically between days 3 and 8 *in vitro* (80307± 2253 v's 147594 ± 1893 d.p.m./well of cells, mean ± s.e.mean n=4 $p < 0.001$, Student's t-test) and continued to increase between DIV15 and DIV18 (156107 ± 5684 v's 185752 ± 1337 d.p.m./well of cells). The uptake mechanism also demonstrated Na^+ dependency, which altered with increasing age in culture (% reduction of uptake due to the absence of Na^+, DIV5 = 23 ± 1.8%, DIV9 = 65 ± 2.2%, DIV15 = 41 ±3.1%, DIV 21 = 50 ± 3.8%). The specific inhibitor of GABA uptake into neurones, SKF 89976 A, used at a range of concentrations (0 to 10^{-3}M), caused a dose dependent reduction in uptake (0 = 80307 ± 2253, 10^{-6}M = 47156 ± 914, 10^{-5}M = 38735 ± 1238, 10^{-4}M = 22371 ± 425.0 and 10^{-3}M = 3101 ± 99 d.p.m./well of cells, on DIV3), the effect becoming less marked with culture age. In contrast, the specific inhibitor of [^{3}H]GABA uptake into glial cells, ß-alanine, exhibited no consistent dose-dependent effect on uptake throughout the period of investigation. Non-specific (56mM K^+) depolarization - induced [^{3}H]GABA release, over a 7 min incubation period, (5923 ± 65 d.p.m./well of cells), was calcium dependent and significantly greater ($p < 0.001$, Student's t-test), than basal release (1173 ± 146 d.p.m./well of cells). A second stimulus with 56mM K^+ produced a response consistently greater than the first (5923 ± 65 v's 100963 ± 507 d.p.m./well of cells $p < 0.001$), which is in opposition to the secretion dynamics for [^{3}H]DA in this preparation where the response to a second stimulus was much reduced (Murray & Gillies 1993).

In conclusion, cultured hypothalamic GABA-ergic neurones demonstrate many ontogenetic and functional characteristics which resemble those found *in vivo* (Johnston & Davies 1974) and further investigations using this model may enhance our understanding of the role played by GABA in the neuroendocrine system.

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Acknowledgements: We are grateful to the Wellcome Trust for financial support of this work.

266P NOVEL ACTIONS OF A BENZODIAZEPINE AND A BARBITURATE ON SOMATOSTATIN RELEASE FROM THE PERINATAL HYPOTHALAMUS IN CULTURE

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In the hypothalamus 80% of neurones possess gamma-aminobutyric acid (GABA) receptors (Ventimiglia et al., 1990) and a growing body of evidence supports a role for GABA in the control of neuroendocrine function. In particular pharmacological manipulations of GABA levels produce marked effects on growth hormone secretion which are thought to be mediated primarily via an action at the hypothalamic level but the mechanism of action remains to be established.

Using hypothalamic cell cultures initiated from rat tissue obtained at embryonic day 18 and maintained in defined medium at a density of 2.4 x 10^6 cells (equivalent to 3 hypothalami) per 35 mm well we have demonstrated a significant intrinsic inhibitory GABAergic tone on the secretion of somatostatin (SRIH) which is the hypothalamic regulatory factor responsible for the inhibition of growth hormone secretion. This action is mediated predominantly via the type A GABA receptor (Gillies and Davidson, 1992). The aim of this present study was to characterise further the $GABA_A$ receptor present in the hypothalamic cultures by investigating the effects of a benzodiazepine (chlordiazepoxide), a barbiturate (phenobarbitone sodium) and a benzodiazepine antagonist (flumazenil) on the release of SRIH. Chlordiazepoxide and phenobarbitone sodium might be expected to enhance the endogenous GABAergic tone on SRIH release via their positive allosteric actions at the $GABA_A$ receptor and therefore reduce peptide release further. However, chlordiazepoxide significantly stimulated SRIH release at 10^{-9} M and 10^{-8} M (70.3 ± 7.19 and 67.5 ± 0.94 pg / hypothalamus respectively with $p < 0.01$ vs basal release of 22.3 ± 2.3 pg / hypothalamus. All values are the mean ± SEM) as did 10^{-8} M phenobarbitone sodium (63.1 ± 3.15 with $p < 0.01$ vs basal release of 35.7 ± 3.65 pg / hypothalamus). Flumazenil alone caused a significant inhibition in SRIH release at 10^{-7}, 10^{-6} and 10^{-5} M (65.7 ± 3.57, 58.7 ± 2.59 and 47.3 ± 2.3 with $p < 0.05$, 0.02 and 0.01 respectively vs basal release of 99.2 ± 7.15, expressed as pg / hypothalamus). These results suggest that the benzodiazepine and barbiturate were exerting a negative allosteric effect at the $GABA_A$/benzodiazepine receptor which differs from their actions at the classical $GABA_A$ receptor found in the adult brain. Significant differences in neonatal vs adult $GABA_A$ receptors have also been observed in hippocampal neurones (Cherubini et al., 1991) supporting the concept that the neonatal $GABA_A$ receptors may differ functionally from that found in the adult.

In conclusion these data provide evidence for the existence of a novel $GABA_A$ receptor in the neonatal hypothalamus which, we propose, could play an important role in the ontogenesis of neuroendocrine function. In addition, the significant effect of flumazenil could indicate the presence of an endogenous ligand for the benzodiazepine/ß-carboline binding site and merits further investigation.

Acknowledgements: We are grateful to the Wellcome trust for financial support of this work

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267P THE EFFECT OF CHLORMETHIAZOLE ON cGMP CONTENT OF RAT CEREBELLAR SLICES

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We have shown that chlormethiazole (CMZ) antagonised the tremor and reduced the elevated cerebellar cGMP content induced by the aspartate releasing agent harmaline (Cross et al, 1993a). We now describe the effect of CMZ on cGMP cerebellar slices following treatment with N-methyl-D-aspartate (NMDA) or sodium nitroprusside.

Slices (500μm) of immature (10d) rat cerebellum were incubated at 37°C for 3 min in Krebs-Henseleit buffer in the presence or absence of NMDA (100μM) or sodium nitroprusside (100μM) and test drugs as appropriate. The tissue content of cGMP was determined using a commercially available radioimmunoassay (Amersham International).

CMZ inhibited NMDA-stimulated cGMP content at high concentrations (Table 1), but did not effect basal cGMP content nor the increase in cGMP induced by sodium nitroprusside. The NMDA response was also inhibited by dizocilpine (10μM).

Table 1 The effects of drugs on cerebellar cGMP content *in vitro*

	cGMP Content ($pmolmg^{-1}$ tissue)		
	None	Chlormethiazole (300μM)	Dizocilpine (10μM)
Basal	0.44 ± 0.07	0.45 ± 0.14	0.44 ± 0.08
NMDA	1.66 ± 0.20	1.10 ± 0.12*	0.56 ± 0.08*
Nitroprusside	8.36 ± 0.52	9.96 ± 1.58	-

Values are mean ± s.e.mean, n=3-10. *p<0.05 v NMDA alone.

The inhibitory action of CMZ (300μM) was prevented by the $GABA_A$ antagonist picrotoxin. CMZ does not act as an NMDA receptor antagonist (Cross et al, 1993b), and the present data suggest that it does not inhibit guanylate cyclase. Thus CMZ inhibited the NMDA response indirectly, possibly through its action at the $GABA_A$ receptor.

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268P HYPOTHERMIC EFFECTS OF NNC-711 AND OTHER GABA UPTAKE INHIBITORS IN MICE

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The effects of γ-aminobutyric acid (GABA) uptake inhibitors on body temperature in male NMRI mice (25±2g) were investigated. NNC-711 (1-(2-(((diphenylmethylene)amino)oxy)ethyl)-1,2,5,6,-tetrahydro-3-pyridine carboxylic acid); CI-966 ([1-[2-[bis 4-(trifluoromethyl)phenyl]methoxy]ethyl]-1,2,5,6-tetrahydro-3-pyridine carboxylic acid) and SKF 100330A (N-[4,4-diphenyl-3-butenyl]-guvacine) were suspended in 5% chremophore and administered intraperitoneally. Rectal temperatures were measured 15 min before injection, immediately after injection, and every 15 min for one hour. Treatment group means (n=8) were compared using ANOVA and Dunnett's test. All 3 GABA uptake inhibitors produced marked dose-related hypothermia in mice at doses similar to those preventing seizures in animal models of epilepsy (Taylor et al., 1990; Suzdak et al., 1992).

Table 1 Effect of GABA uptake inhibitors on body temperature in mice

Dose (mg/kg)	NNC-711	CI-966	SKF100330A
Vehicle	37.3±0.3	37.7±0.2	37.3±0.2
1	35.4±0.3 *	37.4±0.2	37.2±0.2
3	33.6±0.2 *	36.2±0.1 *	35.8±0.1 *
10	33.7±0.3 *	34.7±0.3 *	33.9±0.4 *

Results represent body temperature °C (means ± s.e.mean) 30 min after injection; *P<0.05-v-vehicle-treated group.

The hypothermic effects of NNC-711 (3 mg/kg i.p.) were not blocked by the $GABA_B$ antagonist CGP 35348 (3-aminopropyldietoxymethyl-phosphinic acid; 300 mg/kg i.p.; 15 min pretreatment). At 30 min mean rectal temperatures °C ± s.e.mean of the different treatment groups were: vehicle 37.5±0.3; NNC-711 34.1±0.2 *; NNC-711 plus CGP 35348 34.4±0.2 *. The dose of CGP 35348 used in this study has previously been shown to reverse the hypothermic effects of the $GABA_B$ agonist baclofen in mice whilst having no effect on body temperature when administered alone (Jackson & Nutt, 1991). The results of this study suggest that the decrease in body temperature produced by NNC-711 does not involve activation of $GABA_B$ receptors by elevated endogenous GABA levels. Experiments to investigate the possible involvement of $GABA_A$ receptors in this response are complicated by the hypothermic and convulsant effects of $GABA_A$ antagonists (e.g. bicuculline) when given alone.

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269P DEFICITS IN SPATIAL LEARNING ARE CORRELATED WITH CHANGES IN SENSITIVITY OF FRONTAL CORTICAL NEURONES TO ACETYLCHOLINE AND BICUCULLINE IN AGED RATS

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There is considerable interest in the possibility that neurotransmitter changes are responsible for the behavioural changes that occur during ageing. The present study investigated the effects of age on rats' performance in the Morris water maze and on changes in the sensitivity of cholinergic and GABAergic systems in the frontal cortex. Two groups of Sprague Dawley rats were used: young (3-4 months, n=10) and old (21 months at the start of the experiment, n=17). The rats were trained to find a hidden platform in the maze (2 trials 30 min apart per day for 14 days). The old rats took longer to escape than the young rats [$F(1,25)=29.6$, $P<0.001$], requiring 14 days to reach asymptotic performance compared with 7 days in young rats, but their performance varied widely with some performing as well as young rats while others were severely impaired. Selected rats were placed into one of the following groups according to their performance during the acquisition stage: group 1, the best 5 young rats; group 2, the 5 least impaired old rats (escape latencies shorter than mean escape latency of young rats +1SD); group 3, 5 most impaired old rats (escape latencies longer than mean escape latency of the young rats +2SD). The sensitivity of frontal cortical neurones to ACh (200 mM), carbachol (200 mM), bicuculline (10 mM in 80 mM NaCl) and glutamate (200 mM) administered iontophoretically (30 nA for 20 s) was studied in these 3 groups under urethane anaesthesia (1.5 mg kg^{-1} i.p.). Neurones were considered sensitive when the drug altered firing rate significantly ($P<0.05$, Wilcoxon signed ranks test) either during or within 20 s.

The basal firing rate of frontal cortical neurones of the 2 old groups were similar (2.0 ± 0.2 imp s^{-1} unimpaired and 1.9 ± 0.2 imp s^{-1} impaired) but were slower than the young group (2.7 ± 0.2 imp s^{-1}). A greater percentage of frontal cortical neurones of young rats responded to ACh (52.7%) and to carbachol (57.3%) than in unimpaired old rats (38.2% and 40% respectively) and in impaired old rats (37.5% and 37% respectively) but again there was no difference between the 2 old groups. The percentage of frontal cortical neurones responding to bicuculline increased with age, 61.2% in the unimpaired aged rats compared with 35.2% in the young rats but was significantly greater in the impaired aged rats (83.3%) than in the unimpaired aged rats. The sensitivity of cortical neurones to glutamate was not affected by age. There were significant correlations between the percentage of cortical neurones responding to ACh and bicuculline and several parameters of water maze acquisition during days 7 and 8, but not during days 1 and 2 (before spatial learning had begun) or days 13 and 14 (when spatial learning was complete): in general, water maze performance correlated better with neuronal sensitivity to bicuculline than to ACh.

The decrease in neuronal sensitivity to ACh and carbachol could be due to muscarinic receptor down-regulation and the increase in bicuculline sensitivity to increased GABAergic tone. A change in the relative balance of cholinergic and GABAergic systems might explain the decrease in basal firing rate of frontal cortical neurones and the learning deficits observed in aged rats.

270P QUAIL SELECTED FOR SHORT OR LONG TONIC IMMOBILITY RESPONSES DIFFER IN BENZODIAZEPINE RECEPTOR BINDING AFFINITY: REFLECTION OF ACTIVITY OR FEARFULNESS?

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The tonic immobility (TI) response seen in birds as a result of manual restraint is thought to be an indication of fear, with more fearful animals remaining immobile for a greater duration. Two distinct lines of Japanese quail have been selectively bred for long (LTI) and short (STI) TI responses and have been classified as high and low fear groups respectively (Mills & Faure, 1991). Given the importance of the benzodiazepine receptors in anxiety disorders, the binding properties of these receptor sites in the two lines were investigated.

Saturation binding assays using the ligand [^{3}H] flunitrazepam (conc. range 0.005-16nM), diazepam (3μM) to define non-specific binding and a tissue concentration of 5mg/ml were performed according to the method of Bodnoff *et al.* (1987). Levels of specific binding were expressed as fmol/mg protein (protein assay of Lowry *et al.* 1951) and converted for scatchard analyses to give values for K_D (nM) and B_{MAX}.

		B_{MAX}	K_D	
LTI	(n=8)	2120 ± 135	2.49 ± 0.12**	Data are mean ± s.e.m.
STI	(n=8)	2079 ± 184	1.91 ± 0.11**	** $P<0.01$ analysis of variance

The body weights of the STI line were 10-15% lower than the LTI line and informal observations on catching the animals suggested that the STI line were more active (or reactive) than the LTI line.

Higher binding affinities in the STI line would explain a lower TI duration in response to an endogenous agonist. This would not explain the differences in the levels of (re)activity which, together with the body weights of the lines, are sufficient to seriously question whether the lines have indeed been bred for high and low fear. An alternative is that the lines have been selected for differences in activity and there is an endogenous inverse agonist for the benzodiazepine receptor to which the STI line has greater sensitivity. A third possibility is that the animals may have been selected for their mode of fear expression, rather than the level of fear *per se*. Further behavioural experiments with formal, independent measures of activity and/or other measures of fear are required to resolve this important question. The mechanism underlying the differences in receptor affinity is not known, however, we hypothesise that the lines differ in expression of the sub-units of the $GABA_A$-Benzodiazepine receptor complex which influence fear or, more likely, activity.

These studies were supported by a grant from the A.F.R.C.

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271P INCREASES IN BRAIN PREPROCHOLECYSTOKININ MESSENGER RNA LEVELS IN RATS FOLLOWING ACUTE ADMINISTRATION OF FG 7142

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The brain cholecystokinin$_B$ (CCK_B) receptor has been implicated in various behavioural states including anxiety, panic and satiety. In animal models of anxiety, the CCK_B receptor agonist pentagastrin is anxiogenic whereas CCK_B receptor antagonists such as CI-988 are anxiolytic (Hughes *et al* 1990). It is hypothesised that CCK neurones related to anxiety may only be activated in anxiogenic circumstances (Hughes *et al* 1990). The present experiments were designed to investigate, using *in situ* hybridisation, whether acute exposure to anxiogenic stimuli induces changes in preprocholecystokinin (pCCK) gene expression in rat brain.

Groups of male hooded Long-Evans rats (n=6 per group) received as the anxiogenic stimulus an acute injection of the benzodiazepine receptor inverse agonist FG 7142 (10 mg kg^{-1}i.p. or 20 mg kg^{-1}i.p.) or a 10 min exposure to the elevated plus-maze test of anxiety 10 min following an i.p. saline injection. A saline injected group and a non-injected group served as controls. 6 hours after the treatments animals were killed and *in situ* hybridisation performed on 20 µm brain sections according to the method of Wisden *et al* (1991) using an [^{35}S]-oligonucleotide probe complementary to rat pCCK. Hybridised sections together with calibrated standards were apposed to Kodak X-OMAT film to generate autoradiograms. Optical densities of hybridisation signals were quantified by computer-digitised image analysis.

In control rats the pattern of distribution of pCCK mRNA resembled that reported by other groups (Schiffmann and Vanderhaeghen 1991). Administration of FG 7142 (10mg kg^{-1}i.p.) increased pCCK mRNA in the basolateral amygdala and the CA3 pyramidal cell layer of the hippocampus by 33% and 37% respectively compared to the non-injected control group (basolateral amygdala 366 ± 31 and 275 ± 23; CA3 336 ± 18 and 245 ± 20 ($nCig^{-1}$ ± s.e. mean); $p<0.05$). In larger doses FG 7142 (20mg kg^{-1}) increased pCCK mRNA in the dentate gyrus compared to the group exposed to the plus-maze (387 ± 32 and 254 ± 34 $nCig^{-1}$; $p<0.05$). In contrast, FG 7142 did not alter pCCK mRNA levels in thalamic nuclei. Exposure to the elevated plus-maze did not affect pCCK mRNA levels in any of the brain structures investigated compared to either control group. Similarly, there were no significant differences between the non-injected and saline controls.

These data form preliminary evidence that an acute anxiogenic stimulus can increase pCCK gene expression in a few structures of the limbic system previously implicated in anxiety (Pratt 1992) whereas thalamic structures, in which CCK is also expressed, are unaffected. The relationship between these changes in CCK gene expression and any subsequent change in CCK release requires further investigation.

We thank SHERT for the provision of laboratory facilities and Dr B Morris, University of Glasgow, for expert advice on the *in situ* hybridisation.

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272P ANXIOLYTIC-LIKE EFFECTS OF AMPHETAMINE SULPHATE ON THE ELEVATED PLUS MAZE

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Chlordiazepoxide hydrochloride (CDP) dose-dependently increases the time rats spend exploring the open arms of an elevated plus maze. This effect is said to reflect an anxiolytic effect of the compound as the open arms are 'naturally' aversive to undrugged rats. But, as Lister (1991) points out, in this test it can only be assumed that the drug is having an anxiolytic-like effect if it does not increase locomotor activity.

In the present study male Sprague Dawley rats (250-300g, Bantin and Kingman, Hull, U.K., n=18) were either treated (i.p.) with vehicle (water) or CDP (1.0, 3.0 or 7.5 mg/kg) or the stimulant d-amphetamine sulphate (AMP, 0.375, 0.75 or 1.5 mg/kg). The rats' behaviour was monitored by an image analyser and 'Flexible maze' software (HVS Ltd.) which recorded several behaviours including the time on the open arms (%Time) and three measures of locomotor activity: the total number of arm entries (Total Ents), speed in the closed arms (SPC) and on the open arms (SPO). The results of both experiments are shown in Table 1.

Table 1. Effect of chlordiazepoxide and d-amphetamine sulphate on the elevated maze

	Chlordiazepoxide hydrochloride (mg/kg)				d-Amphetamine sulphate (mg/kg)			
Dose	Vehicle	1.0	3.0	7.5	Vehicle	0.375	0.75	1.5
%Time	19.8±2.0	19.6±2.6	27.3±2.2*	38.5±3.2*	19.3±2.4	22.1±2.4	31.8±3.5*	37.6±4.3*
Total Ents	29.0±1.9	34.0±2.0	44.4±1.6	52.8±3.3	31.6±1.5	31.4±2.0	33.4±2.6*	38.6±2.1*
SPC	6.9±0.4	7.9±0.4	10.5±0.4*	11.1±0.6*	8.1±0.4	8.3±0.5	9.1±0.5	9.7±0.4*
SPO	5.9±0.4	5.9±0.4	7.3±0.3	8.8±1.8*	5.4±0.4	4.7±0.4	5.2±0.4	5.5±0.4

*$p<0.05$ compared to vehicle controls Newman Keuls post hoc test.

Both CDP and AMP dose-dependently increased the time spent on the open arms suggesting an anxiolytic-like effect. However, AMP and, more markedly, CDP also dose-dependently increased locomotor activity. These data question the validity of the elevated plus maze as a test of "anxiety" and suggest that further experiments are needed to assess whether the "anxiolytic" effects of drugs on the elevated plus maze can be dissociated from their stimulant properties.

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273P THE ACTIONS OF THE PUTATIVE ENDOGENOUS CALCIUM CHANNEL LIGAND, PALMITOYL CARNITINE, WITH ACUTELY ADMINISTERED ETHANOL AND IN THE ETHANOL WITHDRAWAL SYNDROME

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Dihydropyridines either increase (antagonists, e.g. nitrendipine, Dolin and Little, 1986) or decrease (agonists, e.g. Bay K 8644, Dolin et al., 1988) the acute effects of ethanol. The antagonists prevent, and the agonists potentiate, the ethanol withdrawal syndrome. We now examine the effects of a putative endogenous calcium channel activator, palmitoyl carnitine (Spedding and Mir, 1987) on the acute effects of ethanol, and on the ethanol withdrawal syndrome.

Groups of 8 TO strain male mice (30-35g) were used. All drugs were dissolved in saline and given i.p. Rectal temperature was measured at 15 min intervals for 1.5h, using a 2.5 g/kg dose of ethanol and 30 min pretreatment with palmitoyl carnitine; saline was used as a control for both drugs. Ataxia was measured, every 30 min, on a rotarod, for 2h after administration of palmitoyl carnitine, with or without ethanol 2.5 g/kg (a 180 sec cut-off point was used). Table 1 shows the temperature and ataxia results, 30 min after the ethanol injections. In the withdrawal study, mice were fed ethanol in a liquid diet (17 - 19 g/kg ethanol per day for 7 days); controls were pair-fed an isocalorific diet without ethanol. Immediately on withdrawal from ethanol the mice were injected with palmitoyl carnitine or saline vehicle, then rated for handling-induced convulsive behaviour every hour for 12h on a scale of 0 to 5 (Littleton et al., 1990). Median handling scores, with interquartile ranges, are shown for 4h and 5h into withdrawal (the time of peak withdrawal severity), in Table 2.

Table 1. P.Carn = palmitoyl carnitine 50 mg/kg

Drug treatment	Time (s) spent on rotorod	Body temperature (°C)
Saline + Saline	180 ± 0	37.6 ± 0.2
Saline + Ethanol	93 ± 33 ¶	35.5 ± 0.4 ¶
P.Carn + Ethanol	14 ± 6 *	32.3 ± 0.6 *
P.Carn + Saline	180 ± 0	35.9 ± 0.5 ¶

Table 2. P.Carn = palmitoyl carnitine 50 mg/kg

Treatment (Chronic/Acute)	4h into withdrawal	5h into withdrawal
Control/Saline	1 (1-2)	1 (1-2)
Ethanol/Saline	2.5 (0-3)	3.5 (1-4)¶
Control/P.Carn	0 (0-1)	1 (0-1)
Ethanol/P.Carn	2 (1-2)	2 (1-3)

Statistics by Mann-Whitney U-test: * $P < 0.05$, compared with ethanol + saline. ¶ $P < 0.05$ compared with saline controls

Palmitoyl carnitine, at 50 mg/kg, potentiated the ataxic effects of ethanol but did not itself cause ataxia at up to 100 mg/kg. At 50 mg/kg it lowered body temperature and potentiated the hypothermic action of ethanol. In these respects palmitoyl carnitine acted like a dihydropyridine antagonist. However, at 50 mg/kg it did not alter ethanol withdrawal seizures. This dose of palmitoyl carnitine, and lower doses, we previously reported to potentiate NMDA seizures (Watson and Little, 1993). It did not therefore resemble either dihydropyridine agonists or antagonists in its effects on ethanol withdrawal.

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274P THE EFFECT OF TWO DIHYDROPYRIDINE CALCIUM CHANNEL ANTAGONISTS ON THE DISTINCTIVE CONVULSIVE COMPONENTS OF ETHANOL WITHDRAWAL

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The ethanol withdrawal syndrome in mice includes changes in sensitivity to different convulsive stimuli with different times courses (Watson and Little, this meeting). Acute administration of the dihydropyridine calcium channel antagonists, isradipine and nitrendipine, reduced the handling-induced convulsive behaviour seen during ethanol withdrawal in mice (Littleton et al., 1990), but the dihydropyridine felodipine did not show this effect (Watson et al., 1992). We now investigate the actions of felodipine and nitrendipine, at doses which caused similar displacement of tritiated-isradipine from the brain in ex vivo binding studies (Watson et al., 1992), on the different convulsive components of ethanol withdrawal.

Male TO mice (20-30g) were administered ethanol by liquid diet (16 - 24 g/kg ethanol per day for 7 days). Control groups received control diet in a pair-fed regime. All mice were withdrawn from ethanol at 09:00 hrs and then injected i.p. with vehicle (0.5% Tween), nitrendipine, 50 mg/kg or felodipine, 10 mg/kg (10-12 mice per group). The measurements were made at the time of maximum change in each parameter during withdrawal. Handling-induced convulsive behaviour scores were rated on a scale of 0 to 5 (Littleton et al., 1990). Audiogenic convulsions were counted after ringing an electric door-bell for 30 sec. The threshold convulsant doses (mg/kg) of bicuculline, aminophylline, or N-methyl-D,L-aspartate (NMDLA) were measured by intravenous infusion, in separate groups of mice.

The elevated handling scores seen in mice withdrawn from ethanol were significantly reduced by nitrendipine, 50 mg/kg, but not by felodipine, 10 mg/kg (medians with interquartile ranges, 3h into withdrawal: controls 0(0-1)*; vehicle 2.5(2-3); nitrendipine 1(0-2)*; felodipine 2(1-3), *$P < 0.05$ Mann Whitney U test cf vehicle group). The audiogenic convulsion incidence at 8h was reduced by nitrendipine but not by felodipine; controls 0/10*; vehicle, 6/10; nitrendipine 1/10*; felodipine 6/10 (*$P < 0.05$ Fisher's Exact Probability test c.f. vehicle). A similar pattern was observed for the aminophylline thresholds, 4h into withdrawal; means + s.e.m.: controls 831 ± 50*; vehicle 1021± 53; nitrendipine 843 ± 67*; felodipine 913 ± 99 (*$P < 0.05$ Student's t-test c.f. vehicle group.). No significant difference were obtained in the studies with NMDLA, although a similar pattern was seen (controls 302 ± 19; vehicle 233 ± 23*; nitrendipine 304 ± 39; felodipine 244 ± 80 (*$P < 0.05$ c.f. controls).

These data demonstrate that injection of nitrendipine on withdrawal from long-term ethanol treatment can prevent or reduce many of the changes during ethanol withdrawal. The decrease in sensitivity to aminophylline might be related to production of adenosine as an endogenous anticonvulsant during the initial stages of ethanol withdrawal. As the handling-induced behaviour was reduced by nitrendipine treatment, this might have resulted in less adenosine being released and thus a lower sensitivity to the infused adenosine antagonist. In summary, nitrendipine reduces the increases in handling-induced convulsions and audiogenic convulsions and it reduces the increased convulsion threshold of aminophylline seen during ethanol withdrawal. These studies also show that felodipine differs from nitrendipine in its actions on each of these measures of ethanol withdrawal severity.

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275P RELEASE OF NEUROTRANSMITTER AMINO ACIDS FROM BASOLATERAL AMYGDALOID COMPLEX *IN VIVO*

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The amygdaloid complex is a highly differentiated structure within the limbic system, which has a multitude of connections with both cortical and subcortical regions (Aggleton, 1991). The basolateral amygdaloid nucleus (BLA) is one of the most widely used structures in electrical kindling and anxiety studies. In the present study we have determined the extracellular levels of excitatory and inhibitory amino acids in the region of the BLA and examined the ability of depolarising agents to release these neurotransmitter candidates in conscious unrestrained rats.

Male Sprague-Dawley rats (290-300g) were implanted bilaterally with guide cannulae aimed at the BLA using stereotaxic surgery. The animals were left to recover for a week before insertion of dialysis monoprobes (Young *et al.*,1988*)*. Probes were perfused with artificial cerebrospinal fluid delivered by a microinfusion pump at 3 µl/min. Potassium (K^+;100mM;10min), or veratridine (Vtd.;100µM;10min) with or without tetrodotoxin (TTX;0.5µM;10min) was applied in the dialysis stream by means of a liquid switch. The amino acid content of the perfusates collected every 10 min were analysed using HPLC. The results are summarised below.

Condition.	Aspartate		Glutamate		Glycine		GABA	
	Left.	Right	Left.	Right	Left.	Right.	Left.	Right.
Basal	2.95±0.98	1.64±0.49	27.22±7.13	19.35±7.13	52.40±18.94	50.02±16.40	3.36±0.33	3.85±0.82
K^+	5.58±1.15	4.10±1.31	154.99±35.80*	230.50±110.6*	83.31±9.51	67.08±23.78	10.33±5.74	10.66±4.34
Basal	3.77±1.07	4.67±1.89	33.13±10.00	26.90±5.87	143.83±39.44	122.34±41.82	8.20±3.28	7.30±2.21
Vtd.	26.40±10.25	40.18±10.25	152.11±45.92	186.37±89.87	293.48±84.38*	249.20±60.68*	11.81±4.92	12.96±3.85
Vtd.+TTX	4.60±0.71	6.14±1.81	50.24±5.26	68.20±16.41	237.10±71.13	218.69±87.488	--	--

Table1. Dialysate amino acid concentrations (nM), measured in the region of the left and right BLA (values are mean ± s.e.mean; n=4). * $P < 0.05$ stimulated compared with basal (matched pairs t-test).

These results support a role for glutamate as an excitatory neurotransmitter in the amygdaloid complex. They also indicate that glycine can be released by depolarising stimulation *in vivo* and may thus serve a coagonist role with glutamate at amygdaloid synapses. Although clear trends towards stimulation-evoked increases in aspartate and GABA were observed these changes were not statistically significant.

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276P CARBACHOL AND *N*-METHYL-D-ASPARTATE EVOKE Ca^{2+} RELEASE FROM DIFFERENT INTRACELLULAR STORES IN CULTURED CEREBELLAR GRANULE CELLS

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Whereas the two main mechanisms of Ca^{2+} release from intracellular stores, inositol-1,4,5-trisphosphate (IP_3) and Ca^{2+}-evoked release, have been characterized in a variety of cell types, the inter-relationship between these phenomena is less well established. In one type of neurone, cerebellar granule cells, it has been proposed that both IP_3 and Ca^{2+} may act on functionally the same Ca^{2+} pool (Irving et al., 1991). In the present study we have investigated Ca^{2+} mobilization evoked by the glutamate receptor agonist NMDA, which activates Ca^{2+} entry, and the cholinoceptor agonist carbachol (CCh), which evokes IP_3 generation in cultured cerebellar granule cells via muscarinic receptor activation (Whitham et al., 1991).

Cerebellar granule cells were cultured from neonatal Wistar rats as described previously (Whitham et al., 1991). For measurements of $[Ca^{2+}]_i$, cells were incubated with 2 µM fura 2-AM for 20 min, and experiments performed on groups of 5-8 cells as described previously (Simpson et al., 1993).

CCh (1 mM) evoked an elevation of $[Ca^{2+}]_i$ which was rapid in onset, and which decayed within 40 s to a very small plateau (peak increase in 340/380 nm ratio 0.98 ± 0.10, plateau increase 0.11 ± 0.02; basal ratio 1.07 ± 0.08). This response was virtually abolished by pre-incubation with the intracellular Ca^{2+} store Ca^{2+}ATPase inhibitors thapsigargin (10 µM) and 2,5-di-(t-butyl)-1,4-benzohydroquinone (BHQ)(25 µM), suggesting that the $[Ca^{2+}]_i$ elevation evoked by CCh is dependent on Ca^{2+} stores. Furthermore, pretreatment of cells with ryanodine (10 µM) or dantrolene (25 µM) also supressed the $[Ca^{2+}]_i$ response to CCh. This indicates that Ca^{2+}-induced Ca^{2+} release is involved in the action of muscarinic agonists, perhaps secondary to Ca^{2+} release from a small IP_3-sensitive pool, or that IP_3 can release Ca^{2+} from stores also sensitive to Ca^{2+}. NMDA (200 µM) evoked a large peak elevation of $[Ca^{2+}]_i$ but, in contrast to CCh, also evoked a large plateau elevation which was maintained throughout the period of agonist stimulation (peak increase 2.03 ± 0.13, plateau increase 1.99 ± 0.11; basal ratio 1.22 ± 0.02). This response was markedly diminished by thapsigargin, ryanodine and dantrolene, demonstrating that NMDA evokes Ca^{2+} release, secondary to Ca^{2+} entry through the NMDA channel (Simpson et al., 1993). However, BHQ had no effect on the NMDA-evoked $[Ca^{2+}]_i$ plateau (105 ± 6% of control).

It appears therefore that in cerebellar granule cells, muscarinic cholinoceptor-stimulated IP_3 generation evokes transient Ca^{2+} release from stores, whereas NMDA receptor-mediated Ca^{2+} entry evokes Ca^{2+} release over an extended time period, from Ca^{2+} stores which can be distinguished from those released by IP_3 by their sensitivty to the Ca^{2+}ATPase inhibitor BHQ.

We thank the Wellcome Trust for the provision of a Prize Studentship in Toxicology to Peter B. Simpson.

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277P PHARMACOLOGY OF METABOTROPIC GLUTAMATE RECEPTOR ACTIVATION OF RAT SPINAL NEURONES *IN VIVO*

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In addition to the well known ionotropic effects, L-glutamate also modulates several aspects of neuronal function via a G-protein coupled metabotropic receptor (mGluR: Schoepp and Conn, 1992). We investigated the effects of mGluR ligands on the activity of spinal neurones in pentobarbitone-anaesthetised rats using iontophoretic administration of compounds.

The prototypical mGluR agonist, 1S,3R-1-aminocyclopentane-1,3-dicarboxylate (1S,3R-ACPD; 50mM in 150mM NaCl; 1-20nA) had little effect on basal firing alone but potentiated the excitatory action of both N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) by 81 ± 18 (mean ± s.e.m.; n = 15) and 76 ± 17% (n = 17) respectively but also enhanced GABA- and glycine-mediated inhibitions. Of the other ACPD isomers, 1S,3S weakly mimicked 1S,3R, 1R,3S was essentially inactive and 1R,3R was an NMDA-like agonist. The effect of 1S,3R-ACPD was mimicked by other mGluR agonists (approx. relative potencies in brackets):- 1S,3R-ACPD (100) > 2S,3S,4S-alpha-(carboxycyclopropyl)-glycine (L-CCG-I; 80) > 3,5,-dihydroxyphenylglycine (70) > quisqualate (20) > L-2-amino-4-phosphonobutyrate (L-AP4; 10).

Carboxyphenylglycine metabotropic antagonists (Jane et al., 1993) were ejected concurrently with 1S,3R-ACPD. (RS)-alpha-methyl-4-carboxy- and (S)-4-carboxy-3-hydroxy-phenylglycine, ejected from a 10mM in 200mM NaCl solution with a current of 10nA, produced a 50% reduction of the excitatory effects of 1S,3R-ACPD described above whereas, ejected with the same current, (S)-4-carboxy-phenylglycine was inactive. L-2-Amino-3-phosphono-propionate (L-AP3; 30-50nA from a 100mM solution) was also without effect.

Our results are in general agreement with those of Bleakman et al. (1992) and further suggest that our observations may be explained by a reduction of a potassium conductance via a cyclase-coupled mGluR.

Acknowledgements: We thank Profs. J.C. Watkins & H. Shinozaki & Mr J. Goldsworthy for gifts of compounds.

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278P EFFECT OF LAMOTRIGINE ON MICRODIALYSATE AMINO ACID CONCENTRATION IN THE STRIATUM AFTER FOCAL CEREBRAL ISCHAEMIA IN THE RAT

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Lamotrigine (3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine) is anticonvulsant in animals and in humans and reduces veratrine-induced glutamate release by a possible interaction with Na^+ channels (Miller *et al*, 1986). We have previously shown that lamotrigine (8-20mg kg^{-1} i.v.) is cerebroprotective after middle cerebral artery (MCA) occlusion in rats (Smith & Meldrum, 1993). Microdialysis probes (2mm) were implanted in the left striatum (from bregma; AP=+0.7; L=+3.5; V=-7) of anaesthetised (2% halothane in 70% N_2O & 30% O_2) male Fischer rats. The probes were perfused (3µl min^{-1}) with modified Krebs ringer solution (mM; NaCl 124, KCl 3.3, KH_2PO_4 1.25, $MgSO_4$ 2.4, $CaCl_2$ 1.6; pH 7.4) for 1.5h, then three 15min baseline samples were collected before MCA occlusion. Glutamate was detected fluorimetrically with OPA by HPLC. Rectal temperature was maintained at 36-38°C. Rats received immediately vehicle (0.25% w/v methylcellulose in H_2O, n=10) or lamotrigine isethionate (20mg kg^{-1}, i.v. over 10min, n=7) after occlusion. Microdialysate samples were collected for a further 5h, then anaesthesia was discontinued. At 24h a neurological test was performed (0 "normal", 1 forelimb flexion, 2 decreased resistance to lateral push, 3 circling) then ten 20µm serial brain sections (at 1mm intervals from the frontal pole) were cut and stained with cresyl violet. Infarct volumes (mean±s.e.mean mm^3) were estimated by image analysis.
Lamotrigine reduced total and cortical infarct volumes from 135±20 to 76±20 mm^3 (P≤0.01) and from 70±10 to 35±13 mm^3 (P≤0.05) respectively but not noncortical infarct volume when compared with vehicle-treated controls (Student's unpaired *t* test). Neurological deficit was improved in the lamotrigine-treated group (vehicle score 2.4±0.2, lamotrigine score 1.7±0.2, P≤0.05).

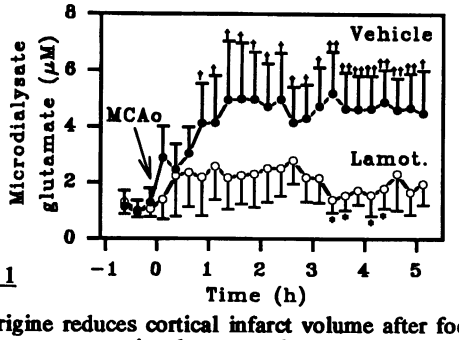


Figure 1

Figure 1 Effect of lamotrigine on microdialysate glutamate concentration in the striatum after focal cerebral ischaemia in the rat.
Rats received (i.v.) vehicle (n=8): closed circles, or lamotrigine (20mg kg^{-1}, n=7): open circles. Microdialysate glutamate concentration increased in the vehicle-treated group after 1h (†: P≤0.05, ††: P≤0.01 ANOVA) compared with basal values. The lamotrigine-treated group did not show a significant increase in microdialysate glutamate concentration compared with basal values. After 3.25h microdialysate glutamate concentration was significantly lower, in the lamotrigine-treated group (*: P≤0.05, ANOVA), than in the vehicle-treated group.

Lamotrigine reduces cortical infarct volume after focal cerebral ischaemia in rats. In the striatum, the reduction in microdialysate glutamate concentration does not relate to cerebroprotection. It is unlikely that the effects of lamotrigine are specific for glutamate.

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279P IFENPRODIL, ELIPRODIL, MAGNESIUM, DEXTRORPHAN AND POLYAMINE SPIDER TOXINS DISCRIMINATE NATIVE NMDA RECEPTOR SUBTYPES IN THE RAT STRIATUM

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NMDA receptor subtypes have different pharmacological properties. Magnesium (Monyer et al,1992) or $Argiotoxin_{636}$ (Raditsch et al,1993) are relatively weak antagonists at NMDA receptors containing NMDAR2C subunits (versus NMDAR2A or NMDAR2B) while ifenprodil is a potent antagonist at receptors containing NMDAR2B with little effect at receptors containing NMDAR2A (Williams et al,1993). We show below that these compounds and also dextrorphan and dextromethorphan have markedly different potencies at native striatal NMDA receptors mediating acetylcholine and spermidine release, suggesting that different NMDA receptor subtypes mediate these responses. In perfused rat striatal slices NMDA (1-1000μM) increases the release of [^{14}C] acetylcholine (ACh) previously synthesised from [^{14}C]choline (2μM, 53 Ci/mmol:NEN) and of [^{3}H]spermidine (SPD,0.4μM,15Ci/mmol: NEN) in a concentration related manner.

	ACh	SPD
MK801	0.21±0.1	0.35±0.2
L-689,560	0.22±0.1	0.27±0.1
CGP37849	23±13	4.8±0.8
$MgCl_2$	200±10	>1000
Ifenprodil	5.4±1.4	>100
Eliprodil	3.2±3.6	>100
$Argiotoxin_{636}$	5.6±5.2	>10
$Philanthotoxin_{433}$	6.3±4	>10
Dextrorphan	1.4±0.1	11±4
Dextromethorphan	12±3	>100

The effects of NMDA (300μM) on both ACh and SPD release are blocked with equal potencies by the channel blocker MK801, the competitive NMDA antagonist CGP37849 (Pozza et al,1990) or the glycine antagonist L-689,560 (Grimwood et al,1993). $MgCl_2$, ifenprodil, eliprodil (SL 82.0715), polyamine spider toxins (generously supplied by Profs Usherwood and Nakanishi), dextrorphan and dextromethorphan selectively blocked the NMDA induced release of ACh without, or weakly, affecting NMDA-evoked SPD release. IC_{50} values (μM) versus NMDA evoked ACh or SPD release are shown on the left. Like ifenprodil or eliprodil, dextrorphan and dextromethorphan are neuroprotective in animal models of ischaemia but relatively devoid of MK801 like side effects in vivo (Tortella et al,1989). NMDA receptor subtype selectivity may explain this profile.

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280P SOMATIC AND DENDRITIC DEGENERATIVE CHANGES INDUCED BY NMDA IN NEONATAL RAT FLEXOR MOTONEURONES *IN VITRO*

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Excessive activation of N-Methyl-D-Aspartate (NMDA) receptors results in excitotoxic cell death in a variety of adult neurons. In motoneurones the density of NMDA receptors is greater in neonates than in adults (Kalb et al, 1992). In this study we have investigated the susceptibility of neonatal motoneurones to the excitotoxic effects of NMDA and the neuroprotective effects of the non-competitive NMDA antagonist dizocilpine maleate (MK-801) using an *in vitro* rat spinal cord preparation. At birth (P0), ankle flexor motoneurones which innervate the tibialis anterior muscle were retrogradely labelled by intramuscular injection of the fluorescent dyes Fast Blue and Diamidino Yellow. At P7, the spinal cord was hemisected mid-sagittally under oxygenated Krebs solution and exposed for 90 minutes to various concentrations of NMDA (5-100 μM) in the presence or absence of MK-801 (4-44 μM). The preparations were fixed and the extent of motoneurone degenerative changes was analysed by: (1) somatodendritic labelling with the carbocyanine dyes DiI and DiA, (2) light microscopy (semithin sections) and (3) electron microscopy (EM).

Exposure to NMDA resulted in degenerative changes throughout the spinal cord, especially in dorsal horn neurones and motoneurones, in a concentration-dependent manner. The first signs of damage in motoneurones, at 10μM concentration, consisted of varicose swellings in distal dendrites. A few motoneurones showed cytoplasmic vacuolation in the soma. At 50-100 μM most motoneurones displayed somatic as well as dendritic vacuolation. At the EM level, there was evidence of dilatation of the endoplasmic reticulum and of mitochondrial damage as well as extensive cytoplasmic vacuolation. In the nucleus, chromatin clumping and nuclear membrane damage was evident. Coincubation of NMDA with its antagonist MK-801 fully prevented the degenerative changes. The most striking difference was the absence of dendritic varicosities. At the EM level, the cytoskeleton, endoplasmic reticulum, and mitochondria had a normal appearance.

These results show that *in vitro* exposure of neonatal motoneurones to NMDA causes concentration-dependent degenerative changes first localized to the dendrites followed by somatic damage. These changes can be fully prevented by the selective antagonist MK-801.

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281P ASCORBATE OR ALLOPURINOL PROTECT AGAINST KAINATE-INDUCED NEUROTOXICITY

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Systemic kainate administration at a dose $\geq 10mg.kg^{-1}$ results in lesions in the hippocampus, entorhinal cortex and the amygdala, but not in the striatum. The mechanism of this damage is thought to involve the release of presynaptic glutamate (Ferkany *et al.*, 1982), which acts postsynaptically to depolarise the membrane and activate the NMDA receptors. There is increasing evidence for the involvement of NMDA receptors in kainate mediated toxicity (Dykens *et al.*, 1987, Ferallio *et al.*, 1989). Following this damage there is a period of reactive gliosis and an invasion of the site of injury by phagocytic cells. We have previously reported that adenosine A_1 agonists/antagonists will modulate the kainate induced elevation in the density of peripheral benzodiazepine receptors (MacGregor *et al.*, 1993). Recently the involvement of free radicals in kainate toxicity has been suggested, and with this in mind we examine the effect of ascorbate and allopurinol on kainate toxicity.

Male Wistar rats (180-220g) were injected with $0.2mg.kg^{-1}$ clonazepam, $10mg.kg^{-1}$ kainate and either drug or vehicle. The animals were killed on the seventh day after injection and the hippocampi removed and a mitochondrial P_2 membrane fraction prepared as in MacGregor *et al.* (1993). The membranes were incubated for 1 hour on ice with 1.75nM [^{3}H]PK11195; the incubation was terminated by vacuum filtration and the filters counted for DPM.

The kainate administration resulted in a an elevation of [^{3}H]PK11195 binding from 81.5 ± 5.1 (n=8) to 251.0 ± 22.9 fmol.mg protein^{-1} (n=6), (mean ± SEM, $p \leq 0.001$ t-test). This increase in binding was significantly reduced in a dose dependent manner by ascorbate, with maximal protection obtained at $50mg.kg^{-1}$ (83.0 ± 8.0 fmol.mg protein^{-1} (n=8) $p \leq 0.001$, t-test). Ascorbate ($50mg.kg^{-1}$) was also administered either before or after kainate treatment, and was only effective either 1 hour before or immediately after the kainate injection (143.14 ± 22.51 (n=8) p=0.036, 85.45 ± 10.64 fmol.mg protein^{-1} (n=9), p=0.002 respectively). Allopurinol was administered at various doses (17.5, 100 or $175mg.kg^{-1}$) simultaneously with kainate and was effective at the higher dose (125.46 ± 21.69 fmol.mg protein^{-1} (n=3), p=0.019). However the increase in protection was accompanied by an increase in mortality.

The action of ascorbate was also examined electrophysiologically using the hippocampal slice preparation. Ascorbate at a dose of 4mM was found to have no effect on either the induction or maintenance of long term potentiation when examined for 120 minutes, but did result in a small but significant reduction in the size of the population spike (1.8 ± 0.2% (n=3)).

The results indicate that kainate may be acting through free radical formation as well as released glutamate to mediate its neurotoxic action. Beal *et al.*, (1988) reported that neither ascorbate nor allopurinol were effective in attenuating the damage resulting from intraventricular administration of quinolinate, at doses effective in the present study. This may indicate that free radical formation is occurring prior to NMDA receptor activation.

DGM is supported by a Glasgow University Postgraduate Scholarship and MJH by the Wellcome Trust.

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282P COMPARISON OF EFFECTS OF ISOFLURANE VERSUS HALOTHANE ANAESTHESIA ON LESION VOLUME IN RAT MODEL OF FOCAL CEREBRAL ISCHAEMIA

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In patients undergoing carotid endarterectomy, isoflurane anaesthesia provides a degree of protection against transient incomplete regional cerebral ischaemia which is not observed with halothane (Messick *et al.*, 1987, Michenfelder *et al.*, 1987). In addition, there is evidence that isoflurane affords some protection in hypoxic and haemorrhagic animal models of global cerebral ischaemia (Newberg & Michenfelder, 1983). The aim of this present study was to determine whether this phenomenon is also observed in a model of focal cerebral ischaemia.

Male Lister hooded rats (250-280g) were anaesthetised with either halothane or isoflurane at an initial concentration of 5% in $95\%O_2\backslash 5\%CO_2$, administered through a loosely fitting mask, and maintained at 2% throughout the surgical procedure. Rectal temperature was maintained at 37°C. Once anaesthetised they were positioned in a stereotaxic frame, and the skull was exposed. A bifurcated fibre optic light guide from a 300W xenon arc lamp was positioned on the skull above the parietal cortex. Rose bengal dye (30mg/kg) was injected into a lateral tail vein. The skull was illuminated for 10 minutes, after which the wound was sutured. Each animal was anaesthetised for a total of 40 minutes. Seven days later the rats were sacrificed and perfusion-fixed with neutral buffered formalin. Serial coronal sections 60µm thick were cut through the lesions and stained with cresyl fast violet. The total area of each lesion was determined by digital planimetry and volumes calculated using Simpson's rule.

Table 1 : Lesion volumes in mm^3 (mean ± SEM) for left and right hemispheres in halothane and isoflurane treated groups (analysis by ANOVA).

	Left hemisphere	Right hemisphere	Mean volume
Halothane	24.62±3.20 (n=10)	17.02±2.71 (n=10)	20.82±2.95 (n=10
Isoflurane	5.54±1.12 (n=10)	3.61±1.02 (n=10)	4.58±1.07 (n=10)
Comparison of treatments	p=0.0001	p=0.0002	p=0.0001

The results (table 1) demonstrate that the size of photothrombotic lesions of the parietal cortex can be significantly affected by the anaesthetic used during surgery. The small lesions seen in the isoflurane anaesthetised animals suggest that the neuroprotection observed with isoflurane relative to halothane in both clinical studies and animal models of global ischaemia was also demonstrated in this model of focal cerebral ischaemia.

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283P FLUNARIZINE FAILS TO REDUCE LESION VOLUME IN A NON-HUMAN PRIMATE MODEL OF FOCAL CEREBRAL ISCHAEMIA

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It has been demonstrated that photochemical induction of a thrombosis produces lesions of reproducible area and depth in the rat cortex (Watson et al. 1985). An advantage of this stroke model is that it is relatively non-invasive which makes it suitable for use in primates. Therefore, this methodology was extended to the common marmoset (Callithrix jacchus), in an attempt to model more closely one of the conditions that gives rise to the human disorder. It has been reported that in rats the calcium channel antagonist, flunarizine, reduces the volume of such photochemically induced lesions (Van Reempts et al. 1987, De Ryck et al. 1989). In this study we describe the effect of two doses of flunarizine on bilateral photothrombotic lesion volumes in the marmoset.

Twenty four adult marmosets of both sexes (290-450g) were anaesthetised with an initial dose of Saffan (alphaxalone/ alphadolone 18mg kg^{-1} i.m.). A flexible cannula was inserted into a lateral tail vein in order to administer both a maintenance dose of Saffan (6mg kg^{-1} i.v.) and the photothrombotic dye, rose bengal. The head was placed in a stereotaxic frame, the skull exposed and a fibre optic light guide (3mm diameter) from a 300W xenon arc lamp aligned on the skull surface 14.5mm anterior to the intraural line (AP zero plane, Stephan et al. 1980) and 2.5mm left of the midline. Rose bengal was administered (20mg kg^{-1}) and the skull illuminated for 5mins. The light guide was then moved 2.5mm right of the midline and the skull illuminated for another 5mins. The scalp was sutured closed and the animals allowed to recover for seven days before being sacrificed by perfusion fixation. Serial coronal sections 60μm thick were cut through the lesions and stained with cresyl fast violet. The total area of each lesion was determined by digital planimetry and volumes calculated using Simpson's rule. Animals were divided into three groups: a vehicle (10% w/v β-hydroxy cyclodextrin in saline) treated group; and two drug (1 or 10mg kg^{-1} flunarizine) treated groups. The animals were dosed i.p. 30mins after ischaemic induction and then twice daily for three days.

The procedure resulted in reproducible lesions confined to the frontal cortex. The results show that flunarizine at either dose failed to influence lesion volume in either hemisphere. Statistical analysis was by analysis of variance.

Table 1 Lesion volumes in mm^3 (mean + s.e.m.) for left and right hemispheres in vehicle and flunarizine treated groups

	Left Hemisphere	Right Hemisphere
Vehicle (n=8)	50.87 ± 3.73	50.42 ± 4.13
Flunarizine 1 mg kg^{-1} (n=8)	53.20 ± 2.42	52.44 ± 2.93
Flunarizine 10 mg kg^{-1} (n=8)	55.62 ± 3.71	53.91 ± 4.91

This study demonstrates the ability of rose bengal to generate reproducible cortical lesions in a non-human primate. However, this dose range of flunarizine did not affect lesion volume, although neuroprotective efficacy has been observed using this model in the rat (Van Reempts et al. 1987, De Ryck et al. 1989). This disparity may be due to species differences and/or our use of a chronic (7 day) point of histological determination compared to an acute (4 hour) endpoint.

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284P DIFFERENTIAL EFFECTS OF DIZOCILPINE AND FLUNARIZINE ON THE SURVIVAL OF RETINAL GANGLION CELLS AFTER INJURY

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One month after optic nerve (ON) transection, approximately 10% of the population of retinal ganglion cells (RGCs) survive in a number of species. It has been reported that some RGCs are present two years after ON transection in the rabbit. These findings raise several questions. For instance, why can some RGCs survive for a long time after axotomy, and are they chemically different from those which degenerate rapidly? Cytochrome oxidase (CO) has been used as a marker for studying neurons which are metabolically highly active. Recently, we have reported (von Bussmann *et al*, 1993) that 4% of the total population of RGCs in the normal rat retina show high CO activity. One month after ON transection, 37% of the CO-positive RGCs in the control retinae survive and, because they stain with CO, must be metabolically active. In contrast, 57% of the CO-rich RGCs survive one month after ON transection and peripheral nerve (PN) graft to the cut ON. Moreover, 77% of the CO-rich RGCs regenerated into the PN graft. This suggests that RGCs which have high oxidative metabolism, as indicated by CO histochemistry, are better able to withstand the trauma of axonal transection and are also likely to regenerate their axons when presented with a permissive environment. The purpose of this study is to investigate the mechanism involved in the degeneration and regeneration of CO-rich RGCs after ON transection.

We used CO histochemistry in combination with different dosages of flunarizine, a voltage-dependent calcium channel antagonist, and dizocilpine, an NMDA receptor antagonist, after ON transection or ON transection and a PN graft. In 27 Sprague-Dawley rats, the left ON was transected. In 3 other rats, a 15 mm segment of the peroneal nerve was grafted to the proximal cut end of the left ON. Thirty minutes before and 30 minutes after surgery, each animal received one dose of either flunarizine (control vehicle, 2.0 mg/kg, 5.0 mg/kg or 10 mg/kg) or dizocilpine (control vehicle, 0.01 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 2 mg/kg), and then one further dose every day until perfusion. After a survival time of 28 days, all the animals were perfused and the retinae removed and processed for staining with methylene blue (MB) or CO histochemistry.

After ON transection, the percentage of CO-rich RGCs which survive ON transection after injections of flunarizine are as follows: control = 34%; 2 mg/kg = 43%; 5 mg/kg = 47%; 10 mg/kg = 67%. After ON transection, the percentage of CO-rich RGCs which survive ON transection after injections of dizocilpine are as follows: 0.01 mg/kg = 19.5%; 0.1 mg/kg = 5.0%; 0.2 mg/kg = 4.5%; 2 mg/kg = 9.5%. After ON transection, the percentage of MB-stained RGCs which survive ON transection after injections of flunarizine are as follows: control = 14%; 2 mg/kg = 14%; 5 mg/kg = 44%; 10 mg/kg = 67%. After ON transection, the percentage of MB-stained RGCs which survive ON transection after injections of dizocilpine are as follows: 0.01 mg/kg = 18%; 0.1 mg/kg = 16%; 0.2 mg/kg = 12%; 2.0 mg/kg = 2.0%.

Our results show that flunarizine greatly increased the number of surviving CO-rich RGCs after ON transection whereas the dizocilpine greatly decreased the number of CO-rich RGCs after ON transection. In the animals which received dizocilpine in addition to a PN graft, the number of CO-rich RGCs was similar to the number of CO-rich RGCs after ON transection only.

von Bussmann, K.A., Garey, L.J., Jen, L.S. (1993) NeuroReport. 4, 247-250.

285P THE EFFECT OF CHLORMETHIAZOLE ON THE DEVELOPMENT OF OEDEMA FOLLOWING A CORTICAL INFARCT

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A focal ischaemic infarct can be induced in rat cortex by administering the photochemical dye Rose Bengal and irradiating the skull with green light (Watson et al, 1985). We recently reported that Evans Blue extravasation occurred after irradiation. The spread of the extravasation, indicating blood-brain barrier breakdown and oedema, was associated with the ischaemic cell death that also occurred (Snape et al, 1993). Both the extravasation and cell death were reduced by chlormethiazole administration.

The current study has measured the effect of chlormethiazole (CMZ) on both the water content (by freeze drying the tissue) and K^+ and Na^+ concentration (by flame photometry) in the infarct area. A photochemical infarct was produced in the right cortex of male Lister Hooded rats (300-400g) as described previously (Snape et al, 1993). Five min later the animals were injected with saline or chlormethiazole (200 $mgkg^{-1}$ i.p.) and killed at various times thereafter. The left cortex was used as control.

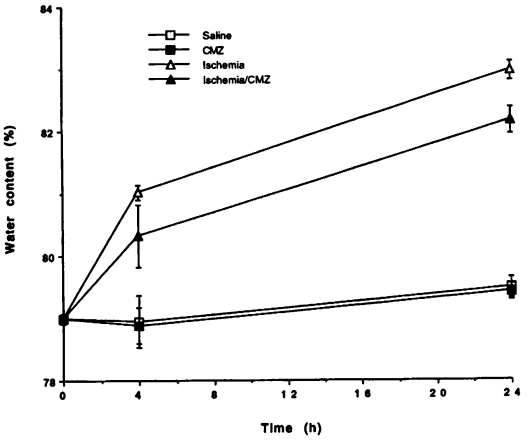


Figure 1. Effect of CMZ on ischaemic oedema

The water content of the ischaemic tissue (right side) increased rapidly following the infarct, the left side being unaffected. Chlormethiazole treatment decreased the oedema (Fig 1, 4h: $p< 0.02$; 24h: $p <0.01$). Ischaemia increased tissue Na^+ content by 61% and decreased the K^+ content by 9%. The Na^+/K^+ ratio therefore increased [control: 0.513 $\pm$ 0.021(5); ischaemic: 0.905 $\pm$ 0.074(10); $p <0.001$]. Chlormethiazole treatment decreased this ratio in ischaemic rats [0.810 $\pm$ 0.080(9); different from ischaemia: $p <0.001$].

The altered Na^+/K^+ ratio suggests the presence of cytotoxic as well as vasogenic odema and the data demonstrate that CMZ has decreased this ischaemic oedema, probably through its previously reported neuroprotective activity.

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286P METABOLITES OF SELEGILINE PROVIDE PROTECTION AGAINST DSP-4 INDUCED DEPLETION OF MOUSE FRONTAL CORTEX NORADRENALINE LEVELS

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Selegiline (SEL; = deprenyl) is an irreversible inhibitor of monoamine oxidase type B (MAO-B) which is used in the treatment of Parkinson's disease as an adjunct to levodopa therapy. However, since SEL can also prevent the activation of the neurotoxin MPTP it has been proposed that it may have a neuroprotective action against endogenous toxins hypothetically causing the neurodegeneration in Parkinson's. SEL can also prevent the depletion in brain noradrenaline (NA) levels after administration of the NA neurotoxin, DSP-4 (Gibson 1987) but this has later been shown to be unrelated to inhibition of MAO-B (Finnegan *et al.* 1990). These latter workers speculated that the protection was due to inhibition of uptake of DSP-4 by the NA transporter. We have now studied the effect of SEL (5 or 10 mg/kg) and its metabolites (all at 5 mg/kg), *l*- desmethylselegiline (*l*- DMS), *l*- amphetamine (*l*- A) and *l*- methamphetamine (*l*- MA) at various times (1-24 h) prior to treatment with a neurotoxic dose of DSP-4 (50 mg/kg, i.p.) in mice. One week later the mice were sacrificed and NA levels in frontal cortex analysed by HPLC-EC.

DSP-4 in saline treated mice (n=25) caused a reduction of 88% in frontal cortex NA concentrations. All the mice treated with SEL or its metabolites 1 h before DSP-4 were protected (frontal cortex values reduced by 25 - 39%, n=5 for each compound). However, neither dose of SEL nor any of the metabolites offered significant protection if 24 h elapsed between treatment and challenge with DSP-4 (NA levels 74 - 85% of control). The longest protection was provided by *l*- MA where up to 12 h could pass between its administration and DSP-4 but NA levels fell only by 45% ($\pm$ 7% s.e. mean; n=10, $P<0.001$ versus DSP-4 alone) in contrast to the other groups i.e. SEL 10 mg/kg (74 $\pm$ 12 % reduction); SEL 5 mg/kg (73 $\pm$ 14%); *l*- DMS 5 mg/kg (78 $\pm$ 13%) and *l*- A (83 $\pm$ 5%).

The results confirm the results of Finnegan et al (1990) that the ability of SEL to protect against DSP-4 induced depletion of central NA levels is unrelated to inhibition of MAO-B (since MAO-B is irreversibly inhibited by SEL). Instead, inhibition of uptake, not only by SEL, but also, and perhaps, via its metabolites, seems to be a likely explanation for this phenomenon.

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287P DISTRIBUTION OF STRIATAL DOPAMINE D_3 RECEPTORS IN THE NORMAL AND MPTP-TREATED COMMON MARMOSET

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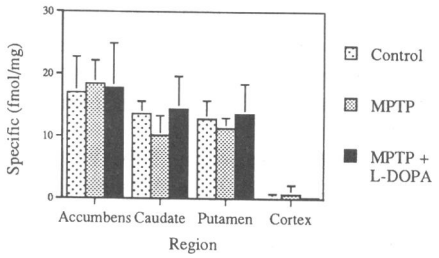
Using the selective dopamine D3 receptor ligand [3H]-7-hydroxy-N,N-di-n-propyl-2-aminotetralin ([3H]-7-OH-DPAT) Lévesque and colleagues (1992) have demonstrated the presence of [3H]-7-OH-DPAT binding sites in the ventral striatum of the rat, namely the shell and anterior part of the nucleus accumbens, olfactory tubercle and islands of Calleja. We now report on the localization of the D3 receptor in the basal ganglia of the normal common marmoset (*Callithrix jacchus*) in comparison to the rat. We also compare [3H]-7-OH-DPAT binding in normal marmosets with that in animals rendered parkinsonian with the dopaminergic neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (5 days, 2mg/kg s.c. dissolved in saline, once daily) and in MPTP treated marmosets treated chronically with L-DOPA and carbidopa (28 days, 12.5 mg/kg p.o. dissolved in 10% sucrose, once daily).

The studies were performed according to the methods of Lévesque and colleagues (1992). High levels of specific [3H]-7-OH-DPAT (1.5nM) binding were found in limbic areas (nucleus accumbens) of the common marmoset. Moderate levels of binding were also seen in caudate and putamen nuclei. No specific binding was found in the frontal cortex. In some sections dense areas of binding were also seen in septal and basal forebrain regions corresponding to the islands of Calleja (Table 1). In the rat high levels of [3H]-7-OH-DPAT binding were seen in the nucleus accumbens shell and islands of Calleja with low levels in the caudate-putamen and cortex. MPTP denervation and treatment with L-DOPA had no significant effect on the levels of [3H]-7-OH-DPAT binding in any of the brain regions examined (Figure 1).

Table 1

		Rat	Marmoset
Specific [3H]-7-OH-DPAT	Accumbens	15.4 ± 2.8	17.0 ± 5.7
(1.5 nM) binding fmol/mg	Caudate	3.6 ± 1.2	13.6 ± 2.0
Values represent mean ± s.d.	Putamen	3.6 ± 1.2	12.8 ± 2.9
(n = 4, *n =2)	Cortex	3.2 ± 1.6	0.0
	Isl. Calleja	53.0 ± 5.0	39.9 & 39.0*

Figure 1 [3H]-7-OH-DPAT Binding to Marmoset Brain
Values represent mean ± s.d. n = 4



These results indicate that the D3 receptor has a more widespread distribution in primates compared to rodents and that dopaminergic denervation has no effect on its density.

Lévesque D. *et al* (1992) *Proc. Natl. Acad. Sci.* USA., 89, 8155-8159

288P DO D_3 RECEPTORS CONTROL THE LIMBIC SEIZURE THRESHOLD?

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The presence of D3 receptors in the nucleus acumbens and subjacent Islands of Calleja (Sokoloff et al., 1990) bears a close resemblance to the striatal dopamine-sensitive anticonvulsant site described by Turski et al. (1988). We have therefore investigated the anticonvulsant activity of apomorphine (a mixed D2/D3 agonist) and three preferential D3 agonists: LY 171555 (Tsuruta *et al.*, 1981), RU 24213 (Euvrard *et al.*, 1980) and 7-OH-DPAT (Levesque *et al.*, 1992), following their discrete microinjection into the limbic forebrain.

Male Wistar albino rats (200-320g) were anaesthetised with halothane and fitted with bilateral indwelling cannulae and allowed 4 days for recovery. Control animals (n=20), injected bilaterally with 1 µl saline in the nucleus accumbens and challenged, 30 min later, with a mixture of 280 mg/kg i.p. pilocarpine and the proconvulsant D_1 agonist SKF 38393 (10mg/kg i.p.; Setler *et al.*, 1978), all developed clonic motor seizures with a mean latency of 33.6 ± 6.3 min. Intra-accumbens pretreatment with apomorphine (20 pmol-6.5 nmol, n=20), LY 171555 (0.2 pmol-7.8 nmol, n=32), RU 24213 (0.2 pmol-7 nmol, n=30) did not reduce the incidence of limbic motor seizures, but all significantly delayed their onset. Minimum effective doses were 2 pmol for RU 24213 (latency 124.8 ± 33.8 min, p=0.0005), 100 pmol for apomorphine (latency 78.2 ± 28.2 min, p=0.04) and 500 pmol for LY 171555 (latency 113.1 ± 26.2 min, p=0.0021) and 7-OH-DPAT (20 pmol-7 nmol, n=26) was without effect.

Control animals injected with 1 µl saline in the Islands of Calleja, required a dose of 300 mg/kg i.p. pilocarpine plus 10 mg/kg i.p. SKF 38393 to produce a 100% seizure rate (mean latency 20.9 ± 5.4 min, n=10). When dopamine agonists were injected into the Islands of Calleja, at a fixed dose of 2 pmol, RU 24213 gave significant protection (latency 77.9 ± 14.2 min, p=0.01, n=20), whilst 500 pmol amounts of LY 171555 (latency 62 ± 36.4 min, p=0.0077, n=6) and 7-OH-DPAT (latency 51.6 ± 37.7, p=0.04, n=6) were required to produce a significant delay in limbic seizure onset. These data show that RU 24213 is by far the most potent anticonvulsant of the D3 agonists tested here. LY 171555 and 7-OH-DPAT also exhibit high affinities for D3 receptors in binding studies, but did not afford protection against pilocarpine-induced seizures, at doses that could be construed to be D3-selective. These findings therefore argue against a specific involvement of D3 receptors in the anticonvulsant action of dopamine.

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289P MUTATION OF SERINE RESIDUES 194 AND 197 IN THE RAT D_2 DOPAMINE RECEPTOR AFFECTS THE BINDING OF ANTAGONIST DRUGS

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A number of serine residues in the fifth trans-membrane domain are conserved in G-protein linked receptors which bind catecholamine ligands but mutagenesis studies of these serine residues in D_2 dopamine receptors have provided conflicting data for the role of these serine residues in the binding of agonist and antagonist drugs (Mansour *et al.* 1992; Cox *et al.* 1992). We have performed site-directed mutagenesis studies on the rat $D_{2(long)}$ receptor; mutating Ser197 to Ala197 (D_2Ala197) and Ser194 to Ala194 (D_2Ala194). The mutant cDNAs were transiently transfected into COS-7 cells using the DEAE dextran technique and the resultant receptors were characterised using ligand binding studies. Saturation binding assays using [^{3}H]spiperone show that wild type and mutant receptors each gave saturable binding levels of around 1pmole/mg membrane protein and wild type and both mutant receptors have a similar high affinity for [^{3}H]spiperone of around 40pM. Competition binding assays using in excess of fifteen antagonist drugs representing a number of different classes (e.g substituted benzamides, butyrophenones, thioxanthines, phenothiazines) were also performed. Most of the drugs tested have an unaltered affinity for the mutant receptors compared with the wild type. However, some drugs have a reduced affinity for either D_2Ala194((-)sulpiride and D0710 about 4 fold) or for D_2Ala197 (raclopride and domperidone about 9 fold). Table 1 shows data for those drugs whose affinity is altered by the Ala-mutant receptors.

Table 1 — Antagonist affinity (Ki[nM] ±s.e.mean)

Antagonist	wild type	D_2Ala194	D_2Ala197
(-)sulpiride	21.7±1.8	83.1±12.9*	22.0±3.4
D0710	4.0±0.1	11.4±0.6*	3.6±0.2
raclopride	3.3±0.1	4.7±0.9	23.1±2.7*
domperidone	3.3±1.1	6.0±0.8	42.1±6.5*

Differences in affinity between wild type and Ala-mutant receptors were significant ($P<0.05$, student t-test) only where indicated by an asterisk. The data are mean±s.e.m., n=3.

Mutation of Ser 194 only reduces the binding affinity of substituted benzamide drugs bearing a sulphonamide moiety. Thus, the amine group on the sulphonamide moiety may form a hydrogen bond with the serine residue. Domperidone and raclopride are the only drugs found to be affected by mutation of Ser197. These drugs have dissimilar structures but contain carbonyl and hydroxyl moieties which could participate in hydrogen bonds to Ser197.

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290P EXPRESSION OF D_2 DOPAMINE RECEPTORS IN THE YEAST *S. POMBE*

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The D2 dopamine receptor is an important therapeutic target being a major site of action of drugs used to treat schizophrenia and Parkinson's disease. In order to study this receptor in isolation we have expressed the receptor in the fission yeast S. pombe. This will also facilitate the isolation of large amounts of receptor for biophysical and structural analysis and help in devising novel systems for screening agonist and antagonist drugs.

The cDNA for the long form of the D2 dopamine receptor (gift from Drs J. Mallet & P.Vernier, CNRS, Gif sur Yvette) was cloned into the vector pREP 1 (gift from Dr. K. Maundrell, Glaxo, Geneva). Competent fission yeast were then transformed with this construct chemically and transformed clones isolated. S. pombe-D2 cells were then grown to mid log phase in selective medium containing thiamine (2μM). Cells were then isolated, washed and transferred to thiamine-free medium to induce receptor expression.Receptors were assayed in cell membrane pellets using the binding of [^{3}H]spiperone with specific [^{3}H]spiperone binding defined as that inhibited by 2μM (+)-butaclamol.

Specific [^{3}H]spiperone binding in the S. pombe cells could be detected following induction , reaching a maximum at about 18 hours when saturation analyses showed Bmax values of 1.36±0.58pmol/mg and Kd values of 272±164 pM (mean±SD, 6 experiments). Competition experiments were performed with a series of drugs selective for dopamine receptors and Ki values are given in Table 1. In each case the competition data fitted well to a single binding site model.

Table 1 Ki values (nM) for the Binding of Ligands to D2 Dopamine Receptors expressed in S. pombe

(+)-butaclamol	7.3±3.9
(-)-butaclamol	3224±1889
haloperidol	23.6±13.3
(-)-sulpiride	147.9±47.4

The data are given as mean±SD and are for three or more experiments.

The data show clearly that an authentic D2 dopamine is being expressed in the yeast cells which retains many of the normal characteristics of the native receptor (high affinity for selective drugs, stereoselective binding of the isomers of butaclamol). The detailed properties of this recombinant receptor are, however, somewhat different from those of the receptor expressed in mammalian cells; the affinities of some of the drugs tested are lower than those seen in the mammalian system. Nevertheless the expression of the D2 receptor in yeast will allow new means of characterisation of the receptor to be developed.

291P LACK OF EFFECT OF D_2 ANTAGONISTS ON THE INTERACTION BETWEEN DOPAMINE AND D_1 DOPAMINE RECEPTORS IN RAT AND BOVINE STRIATA

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It has recently been reported that the non-competitive effect (i.e. a decrease in B_{max}) of the agonist dopamine (DA) on ligand binding to D_2 DA receptors in human putamen could be converted to a competitive effect by pretreatment with a selective D_1 antagonist, and vice versa (Seeman et al., 1989). These data suggest that D_1 and D_2 receptors are linked, possibly at the level of their guanine nucleotide binding proteins. The present study investigated whether such a link could be identified in rat and bovine striatal homogenates.
D_1 and D_2 receptors in rat and bovine striatal tissue were labelled with ^{3}H-SCH 23390 and ^{3}H-spiperone respectively. The selectivities of the D_2 antagonists eticlopride and domperidone were confirmed in displacement experiments; their effects on the interaction between DA and D_1 receptors were then investigated by carrying out saturation experiments in the presence and absence of 2-20 µM DA (Farrell & O'Boyle, 1992) using membranes which had been preincubated with or without 200 nM antagonist for 1 hour at 22°C.
Eticlopride and domperidone showed > 1000-fold selectivity for D_2 vs D_1 sites. The data from the saturation experiments were analysed by LIGAND (McPherson, 1985). B_{max} values (pmol/g) were compared by split-split-plot ANOVA and are summarised in the Table.

		-Eticlopride B_{max}(pmol/g)	+Eticlopride B_{max}(pmol/g)	n	-Domperidone B_{max}(pmol/g)	+Domperidone B_{max}(pmol/g)	n
Bovine	control	42.9±1.9**	43.8±1.9** #	6	41.7±2.3*	44.3±2.9*	4
	2.0 µM DA	32.9±2.1	33.9±2.2	6	-	-	
	20 µM DA	26.1±1.7	32.8±2.8§	6	29.7±2.2	33.6±1.8	4
Rat	control	76.5±4.4**	76.8±4.9**	15	84.6±5.9**	83.3±6.5**	6
	2.0 µM DA	66.5±4.4	69.5±3.9	15	71.4±6.2	73.7±4.4	6
	20 µM DA	58.3±3.5	59.3±3.1	15	61.2±5.7	64.8±6.1	6

*p< 0.05, **p<0.001 for effect of DA

#p<0.05 for effect of eticlopride alone

§p<0.05 vs DA in the absence of eticlopride

DA caused a significant reduction in the density of D_1 receptors in both bovine and rat tissue. Pretreatment of rat striatal membranes with either antagonist, or bovine striatal membranes with domperidone, had no effect on the DA-induced decrease in B_{max}. Pretreatment of bovine striatal membranes with eticlopride caused a small reduction in the decrease in B_{max} induced by 20 but not by 2 µM DA. However, in these experiments eticlopride itself caused a small but significant increase in bovine D_1 receptor density. Neither antagonist reversed the DA effect on B_{max} in either species. Thus, under the conditions described here, the interaction between DA and D_1 receptors remained mixed competitive/non-competitive in nature, providing little or no evidence for the presence of a D_1/D_2 interaction at this level.
This work was supported by EOLAS and the Irish Health Research Board.

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292P DOPAMINERGIC MECHANISMS IN THE ENTORHINAL CORTEX ALTER DOPAMINE EFFLUX IN THE FEMALE RAT NUCLEUS ACCUMBENS AND ARE MODULATED BY OESTROGEN AND PROGESTERONE

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There is increasing evidence to link post-partum psychosis to a dopaminergic dysfunction resulting from the abrupt fall in sex hormones e.g. onset is predicted by an enhanced growth hormone response to apomorphine (Wieck et al., 1991). This study compared the effects of dopamine receptor stimulation in the entorhinal cortex on dopamine release in the nucleus accumbens of ovariectomized (OVX) rats, with and without oestrogen and progesterone priming. Both structures express oestrogen and dopamine receptors (Loy et al., 1988; Mansour et al., 1990) and changes at entorhinal D1 and D2 receptors oppositely affect dopamine metabolites in the nucleus accumbens (Louilot and Le Moal, 1991).

Female Sprague-Dawley rats (250 g) were anaesthetized, ovariectomized and guide cannulae positioned into the entorhinal cortex and nucleus accumbens of one hemisphere. Dialysis experiments were performed using a cross-over design, in control and hormone-primed (17-β-oestradiol, 10 µg s.c. in sesame oil 72 h and 48 h before experiment and progesterone, 500 µg s.c. 5 h before experiment) rats at an interval of 1 week. Prior to experiment, a dialysis probe was positioned in the nucleus accumbens and connected to an HPLC system (Eicom, Kyoto) with an Eicompak CA-5ODS column for dopamine separation.

Apomorphine (10 µg in 0.5 µl) injected into the entorhinal cortex via the guide cannula, reduced the amount of dopamine in dialysates of control OVX rats by a maximum of 15.0 ± 3.8% at 40 min with recovery in 2 h and this reduction increased to 25.4 ± 3.1% in primed animals with only partial recovery at 2 h ($P<0.0001$, n=14). SKF38393 (10 µg in 0.5 µl), a D1 agonist (Sibley et al., 1982), had the opposite effect increasing the amount of dopamine in the dialysates of control OVX rats by 22.7 ± 3.5% at 40 min, an effect which was completely abolished in primed animals ($P<0.0001$, n=12).

The results show combined administration of oestrogen and progesterone to influence dopaminergic mechanisms in structures which might, because the temporal region has been implicated in psychoses, be expected to be involved in post-partum illness in man.

The authors wish to thank the Satoh Foundation and Wellcome Trust for financial support.

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293P CLOZAPINE HAS ELEVATED AFFINITY FOR HUMAN FRONTAL CORTICAL DOPAMINE D_2-LIKE RECEPTORS

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The mechanism of clozapine's unique antipsychotic action remains to be elucidated, although positron emission tomography has indicated that it does not have the high affinity for striatal dopamine D2 receptors shared by all other commonly used antipsychotic drugs. Molecular biology has recently identified two further sites of potential importance to a dopamine receptor mediated action of clozapine. One is the D4 receptor, which is expressed to a greater extent in extra-striatal (limbic and cortical) than in striatal regions, another is the D2B (short) isoform of the D2 receptor, also expressed in such regions of low dopaminergic innervation. Both receptors reportedly have a higher affinity for clozapine than does the striatal D2A (long) receptor (Van Tol et al., 1991; Malmberg et al., 1993).

We have undertaken a study to determine clozapine's relative affinity to human cortical dopamine D2-like receptor sites. [^{125}I]iodosulpride, known to bind to D2 and D3 receptors, was used to define these receptors by ligand binding filtration assay in brain tissue taken post-mortem from human frontal cortex (BA 10) and caudate nucleus, and rat frontal cortex and striatum. Following two centrifugation steps at 48,000g, brain homogenate was incubated at 80-fold dilution at 30°C for 30 mins in 50mM Tris HCl (pH 7.4) containing (mM) NaCl (120), KCl (5), $CaCl_2$ (1), $MgCl_2$ (1), ascorbate (5.7) and radioligand at 0.15nM. Sulpiride at 10μM defined non-specific binding. Displacement curves were generated in triplicate for each tissue with clozapine (3nM-10μM) and the classical antipsychotic fluphenazine (0.1nM-300nM).

Table 1. Displacement of binding to D2-like receptors in human brain.

	Frontal cortex	Putamen		
Clozapine (n=8)	7.40 ± 0.28 (0.73)	6.90 ± 0.14 (0.87)	p<0.001	Data expressed as mean ± s.d. pIC50 values.
Fluphenazine (n=4-5)	8.76 ± 0.15 (0.65)	8.76 ± 0.42 (0.91)		Mean Hill coefficient in parenthesis.

Table 1 shows that while fluphenazine exhibits no difference in its affinity for iodosulpride binding sites between cortex and striatum of human brain, clozapine has a highly significant increase in affinity for the frontal cortical sites over the putamen. This effect was not observed in rat brain where no difference between the affinities for cortex or striatum was observed with either drug. Thus for clozapine the pIC50 values were 6.90 ± 0.34 (cortex) and 6.83 ± 0.12 (striatum). These results indicate clozapine to have a selectivity for human frontal cortical dopamine D2-like receptor sites. This would be consistent with the presence of the D2B subtype in human cortex; the Ki for clozapine at the cloned human D2B receptor is reportedly 35nM (Malmberg et al., 1993) which compares favourably with the approximate Ki of 40nM calculated from the present study. These results indicate a potential site of action for the antipsychotic efficacy of clozapine.

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294P INVOLVEMENT OF D_2-LIKE RECEPTORS IN THE MODULATION OF PREPULSE-INHIBITION (PPI) IN RATS

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Prepulse inhibition (PPI) refers to the inhibition of a startle reflex by the presentation of a weak auditory prepulse immediately before the startle stimulus. Because schizophrenics may show impaired PPI there has been a considerable amount of interest in this paradigm as a potential model for schizophrenia. In accordance with the dopamine (D) hypothesis, pretreatment with direct D agonists such as apomorphine produce a marked disruption of PPI in rats (Swerdlow et al, 1991). In the present study we have examined a) a range of D agonists against PPI, and b) various neuroleptics against the disruption of PPI produced by apomorphine.

Male, Wistar rats (250-350g, Glaxo bred) were used throughout. Before drug testing, the animals were each given a sham (saline) injection and pre-exposed to a startle session. A typical session consisted of 12 presentations of 4 trial types: i) no stimulus, ii) prepulse only (75dB), iii) startle pulse only (118dB), iv) prepulse-pulse (interval between prepulse and pulse = 100ms). Trials were presented in a random sequence with a 20-40s interval between each. The background noise level was 70dB. Prepulse inhibition was defined as the percentage of the startle response under the prepulse-pulse trial compared to the pulse alone trial. Following this preexposure, the animals were used in no more than two studies, each separated by a period of 5-7 days. All experiments were conducted according to a between-subjects design. Pretreatment times for agonists were 10min, antagonists 30min, before test.

Apomorphine (0.1-1mg/kg sc, n=16 per dose) produced a significant disruption of PPI in Wistar rats, with no significant effect on startle amplitude (e.g. PPI: vehicle=53±6%, apomorphine 1mg/kg 9±9%; p<0.01). The mixed D_2/D_3 agonists quinpirole (0.1-1mg/kg ip) and 7-hydroxy-2-(di-n-propylamino)tetralin (7-OH DPAT; 0.03-1mg/kg sc) reduced both PPI and startle amplitude. The D_1/D_5 agonist SKF38393 (0.5-8mg/kg sc) (Pendleton et al, 1978) was ineffective. The disruption of PPI produced by apomorphine (0.5mg/kg sc) was antagonised by haloperidol (0.1-0.3mg/kg sc), raclopride (0.3mg/kg sc) and risperidone (1mg/kg sc) pretreatment. Clozapine was ineffective across a wide dose range (0.0001-5mg/kg sc). At 10mg/kg clozapine produced a marked disruption of startle responding and consequently PPI. At the doses and prepulse parameters used, there was negligible effect of the antagonists alone on PPI. It is concluded that direct D agonists such as apomorphine produce a robust disruption of PPI which may be blocked by antagonists having high affinity for the D_2/D_3 subtypes. The failure of clozapine to reverse this D agonist-induced disruption may be related to its relatively weak affinity for D_2/D_3 receptors (e.g. Seeman, 1992).

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295P THE EFFECTS OF NITRIC OXIDE SYNTHASE INHIBITORS AND OXYHAEMOGLOBIN ON RAT STRIATAL DOPAMINE OVERFLOW USING FAST CYCLIC VOLTAMMETRY

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Recent evidence suggests a role for nitric oxide (NO) in the control of dopamine release in the striatum (Zhu & Luo, 1992; Lonart *et al.*, 1993). In this study we have examined the effects of nitric oxide synthase (NOS) inhibitors and oxyhaemoglobin on native dopamine overflow from rat striatal slices using fast cyclic voltammetry. The aim of the study was to determine the effects of reducing any NO present in the slice on dopamine overflow.

Brain slices (350μm) containing the striatum were prepared from male AHA Sprague-Dawley rats (150-200g). Slices were placed in a perspex bath superfused with oxygenated artificial CSF at 32°C (flow rate = 1.2ml min^{-1}). At least 45min was allowed to elapse before implantation of electrodes. Dopamine overflow following single pulse stimulation (0.1msec, 20V) every 2min was monitored as described by Palij and colleagues (1990). Under these conditions the stimulated dopamine overflow was steady for at least 2-3h. Drugs were applied to the perfusion medium at intervals during this period.

The NOS inhibitors caused a decrease in the size of the electrically stimulated voltammetry signal, a quantitative measure of dopamine overflow. A single concentration (100μM) of N^G-nitro-L-arginine (L-NoArg) elicited a significant decrease (20±5%, $p < 0.05$ ANOVA, n=5) in dopamine overflow from baseline levels, equilibrating within 15-20min period. A cumulative concentration-effect study with L-NoArg (1-300μM; n=4) showed that the highest concentration produced a 39±7% decrease in dopamine overflow from baseline levels. A single concentration (10μM) of 7-nitroindazole (7-NI) also produced a significant decrease (60±4%, $p<0.05$ ANOVA, n=4). 7-NI (0.1-30μM; n=3) produced its effects more rapidly than L-NoArg (approx. 10min) and was more effective (maximum decrease at the highest concentration 79±5%). However, 7-NI at concentrations greater than 10μM markedly affected the voltammogram, making interpretation difficult. The effects of both NOS inhibitors were only partially reversible upon washing for periods of up to 1h. Oxyhaemoglobin (3μM and 10μM) produced a decrease (7±5% and 35±5%* respectively. *$p<0.05$ ANOVA, n=4) in dopamine overflow from baseline levels. The effect of oxyhaemoglobin peaked in about 10-20min and could be reversed by washing. The presence of 1μM (±)-sulpiride did not interfere with the effects of NOS inhibitors or oxyhaemoglobin on dopamine overflow, indicating that the inhibitory effects seen did not involve dopamine D_2 autoreceptors.

The NOS inhibitors used in this study and oxyhaemoglobin all caused a decrease in electrically stimulated dopamine overflow. This suggests that endogenous NO facilitates the electrically stimulated release of dopamine in the striatum.

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296P OMD INHIBITION OF L-DOPA AUGMENTED DOPAMINE RELEASE *IN VITRO*

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Studies in man suggest that elevated brain and plasma levels of 3-O-methyldopa (OMD, the o-methylated metabolite of L-dopa) could reduce the effectiveness of L-dopa in Parkinsonian patients under chronic treatment (Tohgi et al, 1991 & Boomsma et al, 1989). Since this is not thought to result from competition for L-dopa entry into the brain we have used rat striatal brain slices to investigate the effects of OMD on the actual synthesis and release of dopamine (DA) after L-dopa.

Male Sprague-Dawley rats (200–250g) were killed with halothane, their brains dissected out and 500μm slices prepared from both striata with a McIlwain tissue chopper. Two slices of striatum were transferred to a perfusion chamber and continuously perfused at 100μl/min for 30 minutes before experimentation with artificial CSF (mM: NaCl 137, KCl 2.68, $CaCl_2$ 2.20, $MgCl_2$ 1.04, Na_2HPO_4 0.42, D-glucose 10 and $NaHCO_3$ 11.9 prewarmed to 35°C and aerated with 95%O_2 and 5% CO_2). Superfusates were collected into cooled MCC tubes containing 20μl of 1M $HClO_4$ at 10-minute intervals and assayed for dopa, DA, DOPAC and OMD by HPLC with electrochemical detection. After two basal samples, 10μM L-dopa was added to the perfusate for 20 minutes before the slices were depolarised by 40mM KCl for 10 min. At the end of each perfusion, 50 min after the addition of dopa, the two slices in each chamber were weighed, homogenised in 0.5ml 1M $HClO_4$ and the contents of dopa, DA and DOPAC estimated.

The addition of exogenous L-dopa (10μM) significantly increased the spontaneous efflux of DA (from 0.13±0.05 to 1.17±0.41 pmole/mg wet weight of tissue in 10 min, $p<0.05$, n=8) and also the maximum KCl-evoked overflow (to 5.13 ±0.58 from 2.02±0.43 without L-dopa, $p<0.01$, n=8). The spontaneous efflux of DOPAC was also increased significantly ($p<0.01$) but there was no further increase after high K^+. When dopa was given in the presence of 50μM OMD (a concentration which in ratio to 10μM L-dopa is comparable to that found in the plasma of Parkinsonian patients on dopa), it had a smaller (but not significantly different) effect on the spontaneous and evoked efflux of DA than when given alone. The total efflux of DA during the overall superfusion time, including depolarisation was, however, significantly lower than in the absence of OMD ($p<0.05$). OMD did not modify the effect of dopa on DOPAC efflux and although the overall time course of dopa efflux was higher in the presence of OMD this only reached significance 20 min after the addition of dopa. Analysis of tissue content after superfusion revealed that the levels of dopa and DA (but not DOPAC) in slices perfused with L-dopa in the presence of OMD were significantly higher ($p<0.01$ & $p<0.05$ respectively) than in those perfused with L-dopa alone.

These results confirm that superfusion of striatal slices with L-dopa elevates tissue content of DA as well as increasing its spontaneous and depolarisation-induced release (Snyder & Zigmond, 1990). The fact that OMD significantly reduced the efflux of DA whilst still increasing the amount left in slices after L-dopa superfusion suggests that OMD reduces the release of DA formed from exogenous L-dopa. This may contribute to the loss of efficacy of dopa in clinical patients undergoing long-term therapy if intraneuronal synthesis of DA is important.

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297P EFFECT OF MK-801 ON DOPAMINE RELEASE IN THE RAT NUCLEUS ACCUMBENS DURING A CONDITIONED EMOTIONAL RESPONSE TO A CONTEXTUAL CUE

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A conditioned emotional response (CER) is a form of association learning for which there is evidence for the involvement of nucleus accumbens (N.acc). A response is caused when an animal is given a conditioning (eg tone) stimulus previously paired with footshock (the explicit cue) or simply placed in the environment where the footshock was given previously (contextual cue) (Selden *et al.*, 1991). A previous study has shown that CER using an explicit cue is associated with increased dopamine (DA) release in the N.acc (Joseph *et al.*, 1991). The aim of the present study was to determine the effect of a contextual cue on DA release in the N.acc and the involvement of NMDA receptors in the response.

Lister hooded male rats (250-300g) were prepared for *in vivo* microdialysis combined with HPLC with electrochemical detection (Wright *et al.*, 1992) to measure extracellular DA in the medial N.acc. Dialysis probes (concentric) were implanted under halothane anaesthesia one day before behavioural testing. Dialysis samples were collected every 20 min in the home cage from 1-2 h prior to giving footshock (0.5 mA for 1 sec x 10, 1 min apart) with tone (10 sec x 10, 1 min apart) in a testing box. 2 h later, rats were exposed to the tone alone in their home cage and 2 h after the rats were returned to the test box without giving either footshock or tone. Dialysis samples (20 min) were collected throughout the experimental period. MK-801 (5×10^{-5} M at flow rate of 1 µl/min), an NMDA antagonist, was administered into the N.acc, through the dialysis probe, 100 min before the final exposure of the rats to the test box.

Inescapable mild footshock paired with tone produced a delayed increase in extracellular DA (max 135 ± 4% n=8 $p<0.001$) compared with the pre-shock values which lasted up to 40 mins. CER using tone alone (explicit cue) after footshock caused a small, but not significant, increase in extracellular DA (114 ± 11% n=6) while the use of a contextual one (10 min in the test box 4 h after footshock) caused an immediate increase in DA which lasted for 80 min (Table 1). MK-801 administered into the N.acc had no significant effect on the immediate increase in DA in response to the contextual cue but completely prevented the later increase (Table 1).

Table 1: Conditioned DA release in the medial N.acc in response to a contextual cue and the effect of intra N.acc MK-801 (5×10^{-5} M) given 100 min before the cue. Data expressed as a percentage of the value of the last sample before the contextual cue.

Time (min)		0 - 20	20 - 40	40 - 60	60 - 80	80 - 100
Contextual cue	n=6	128 ± 6*	133 ± 7**	125 ± 7**	117 ± 5*	108 ± 8
MK-801 + cue	n=6	120 ± 5*	100 ± 6	102 ± 4	103 ± 7	97 ± 10

* $P \leq 0.05$. ** $P \leq 0.01$ compared to respective control animals (same procedure, but no footshock).

These data indicate that glutamatergic inputs to the medial N.acc, the main source of which are from the hippocampus and prefrontal cortex, may regulate the delayed phase of conditioned DA release, in response to contextual cues, through activation of NMDA receptors in the N.acc.

We thank the Wellcome Trust for support.

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298P THE EFFECTS OF WAY-100635, A POTENT AND SELECTIVE 5-HT_{1A} RECEPTOR ANTAGONIST, ON FEEDING AND 8-OH-DPAT-INDUCED FEEDING IN THE RAT

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WAY-100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride), an analogue of the recently reported 5-HT_{1A} receptor antagonist, WAY-100135 (Fletcher et al., 1993), exhibits potent antagonist activity, without evoking an agonist response, at both somatodendritic 5-HT_{1A} autoreceptors and postsynaptic 5-HT_{1A} receptors (Fletcher et al. and Gurling et al., this meeting). We have studied the effect of WAY-100635 on feeding in the rat, under conditions of low and high basal intake, and on 8-OH-DPAT-induced feeding, a response mediated by somatodendritic 5-HT_{1A} autoreceptors (Hutson et al., 1988).

The consumption of a standard diet (CRM pellets) was assessed immediately after dosing (s.c. in all cases) in free-feeding rats ($n \geq 8$) maintained in their home cages. In separate experiments the effects of WAY-100635 (0.003 - 1 mg/kg) or vehicle were measured during a) daytime feeding (low basal intake), b) night-time feeding (high basal intake) and c) following 8-OH-DPAT (0.1 mg/kg) or vehicle. In a further study, satiety sequence analysis (Antin et al., 1975) was used to score the behaviour of satiated rats (as either feeding, active, grooming, standing or resting) while having access to a weighed quantity of a familiar mash (equal parts pellets/water). These assessments were carried out for each subject every 15s for 1 h commencing immediately after dosing. The amount of mash eaten during the test session was also recorded. The data are reported as the mean food intake ± s.e.m., with dose (mg/kg) given in brackets.

WAY-100635 (0.003-1.0 mg/kg s.c.) had no effect on the food intake of rats under either high (night time intake, veh: 6.0 ± 0.8 g) or low (day time intake; veh: 0.6 ± 0.2 g) baseline conditions. The increase in pellet consumption (0-3 h post drug) induced by 8-OH-DPAT (veh+veh: 0.7 ± 0.2 g; veh+DPAT 2.4 ± 0.4 g) was abolished by WAY-100635 (0.03 mg/kg, 635+veh: 1.0 ± 0.4 g; 635+DPAT 0.9 ± 0.3 g). Similarly, satiety sequence assessment revealed that the increase in mash consumption measured following 8-OH-DPAT (0.1mg/kg) administration was blocked by 0.1mg/kg of WAY-100635 (veh+veh: 4.2 ± 0.8 g; veh+DPAT 7.1 ± 0.8 g; 635+veh: 4.5 ± 0.7 g; 635+DPAT 3.3 ± 0.6 g). Using satiety sequence analysis of feeding behaviour, WAY-100635, per se, did not affect the incidence of any of the behaviours measured but significantly antagonised the increased incidence of feeding behaviour seen with 8-OH-DPAT ($P<0.01$).

These results provide a clear demonstration of the *in vivo* antagonism of a somatodendritic 5-HT_{1A} receptor-mediated response by the potent and selective 5-HT_{1A} receptor antagonist, WAY-100635. WAY-100635 represents an advance over recently described prototype 5-HT_{1A} receptor antagonists in that it is considerably more potent and selective (Fletcher et al., this meeting) than any compound used previously.

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299P EFFECTS OF ACUTE AND CHRONIC TREATMENT WITH THE SELECTIVE 5-HT_{1A} RECEPTOR ANTAGONIST WAY-100635 ON HIPPOCAMPAL 5-HT RELEASE *IN VIVO*

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WAY-100635 (N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridynyl)cyclohexanecarboxamide trihydrochloride, Cliffe, 1993) is a selective 5-HT_{1A} antagonist which has been shown to have greater potency than the recently published 5-HT_{1A} receptor antagonist (S)-WAY-100135 (Fletcher *et al.*, 1993) both *in vitro* and *in vivo* (Fletcher *et al.*, this meeting). In the present study we examined the effects of WAY-100635 on 5-HT release in the rat hippocampus using *in vivo* microdialysis. Additionally, we investigated the effect of chronic administration of WAY-100635 to determine whether prolonged treatment with a 5-HT_{1A} receptor antagonist can induce changes in somatodendritic 5-HT_{1A} receptor mediated responses.
24 hours after implantation of guide cannulae into the ventral hippocampus of anaesthetised male Sprague-Dawley rats (280-350g), dialysis probes were inserted for measurement of extracellular 5-HT (Routledge *et al.*, 1993). Probes were perfused with artificial cerebrospinal fluid pH7 (1.0µl/min) and following a 3h stabilisation period samples were collected at 20 min intervals. 3 control samples were taken prior to s.c. administration of saline, 1 ml/kg, antagonist or 8-OH-DPAT. Samples were collected for a further 3h. Administration of antagonist or vehicle was followed 30 min later by 8-OH-DPAT (0.1 mg/kg s.c.) or saline (1 ml/kg s.c.) and samples were collected for a further 2.5h. In chronic treatment studies, WAY-100635 was administered at a dose of 1 mg/kg s.c. b.i.d. for 14 days. Surgery was performed on the last day of dosing and the experiment performed 24h after the last injection.
The 5-HT_{1A} receptor agonist 8-OH-DPAT (0.1 mg/kg s.c.) significantly ($p<0.05$) decreased hippocampal 5-HT release to 19.2 ± 9.9% of preinjection control values (20-60 fmol / 20 µl dialysate); in contrast, WAY-100635 (1 mg/kg s.c.) had no significant effect on 5-HT release. Pretreatment with WAY-100635 (0.1 - 1 mg/kg s.c.) blocked the inhibitory effect of 8-OH-DPAT on extracellular levels of 5-HT with a minimum effective dose of 0.1 mg/kg s.c. In comparison, the minimum effective dose of (S)-WAY-100135 was previously demonstrated to be 3 mg/kg s.c. (Routledge *et al.*, 1993).
Chronic administration of vehicle or WAY-100635 had no effect on the absolute levels of 5-HT in hippocampal dialysates. Chronic treatment with vehicle had no effect on the ability of 8-OH-DPAT (0.05 mg/kg s.c.)to decrease hippocampal 5-HT release; levels of 5-HT in the dialysates were decreased to 34.6 ± 8.9% of preinjection control values ($p<0.05$). Similarly, administration of the same dose of 8-OH-DPAT to animals previously treated with chronic WAY-100635 elicited a response which was not different from animals treated chronically with vehicle; dialysate 5-HT levels decreased to 23.7 ± 7.1% of preinjection control values ($p<0.05$).
These data provide *in vivo* neurochemical evidence that WAY-100635 is a potent antagonist at the somatodendritic 5-HT_{1A} receptor and is devoid of agonist properties. Chronic administration of WAY-100635 had no effect on the ability of 8-OH-DPAT to decrease 5-HT release, suggesting that 5-HT_{1A} receptor antagonists administered for a period of two weeks do not evoke changes in responses mediated via somatodendritic 5-HT_{1A} autoreceptors.

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300P PAPP, A 5-HT_{1D} RECEPTOR LIGAND, IS NOT A 5-HT AUTORECEPTOR AGONIST OR ANTAGONIST IN THE GUINEA-PIG FRONTAL CORTEX

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It has previously been reported that PAPP (1-[2,(4-aminophenyl) ethyl]-4-(3-trifluoromethylphenyl-O-piperazine) has affinity for the 5-HT_{1D} binding site in calf caudate membranes measured by displacement of [^{3}H]-5-HT in the presence of 8-OH-DPAT and mesulergine to mask 5-HT_{1A} and 5-HT_{1C} sites (Schoeffter & Hoyer, 1989). In this study, PAPP was also shown to inhibit 5-HT_{1D}-linked adenylyl cyclase in the same tissue. In the present study we have compared the affinity of PAPP for the 5-HT_{1D} binding site in the guinea-pig frontal cortex using the selective radioligand [^{125}I]-S-CM-GTNH_2 (serotonin-O-carboxymethylglycyl[^{125}I]-iodotyrosinamide)(Bruinvels et al., 1991) with the effect on the 5-HT autoreceptor in the same tissue.
Radioligand binding studies were performed in membranes from guinea-pig frontal cortex using [^{125}I]-S-CM-GTNH_2 (20pM) as described previously, (Bruinvels et al, 1991). Non-specific binding was determined in the presence of 10µM 5-HT. The K^+-evoked release of [^{3}H]-5-HT from slices of guinea-pig frontal cortex was performed as described previously for rat brain slices, (Ennis &Cox 1982). All the superfusion experiments were performed in the presence of fluvoxamine (1µM).
The affinity of PAPP for the 5-HT_{1D} binding site compared with a number of other ligands is shown in Table 1. The pIC_{50} of 7.95 obtained is in good agreement with that of Schoeffter & Hoyer 1989. Sumatriptan produced a concentration related inhibition of K^+-evoked release of [^{3}H]-5-HT with a pD_2 value of 6.78 ± 0.09. The concentration-effect curve to sumatriptan was shifted to the right in the presence of methiothepin 3µM with an apparent pA_2 of 6.8. This rather low pA_2 value for methiothepin to block sumatriptan is in close agreement with that reported previously (Wilkinson & Middlemiss, 1992) who suggested a possible heterogeneity in the receptor mediating inhibition of 5-HT release in the guinea-pig hippocampus. PAPP in concentrations up to 10µM had no significant effect on K^+-evoked [^{3}H]-5-HT release and did not shift the concentration-effect curve to sumatriptan (pD_2 = 6.71 ± 0.25).

Table 1. pIC_{50} values for displacement of [^{125}I]-S-CM-GTNH_2 and pD_2 values for inhibition of [^{3}H]-5-HT release (n=4)

	pIC_{50}	sem	pD_2	sem
5-HT	8.18	0.10	7.4	0.3
5-CT	9.05	0.03	8.4	0.2
Sumatriptan	7.28	0.05	6.8	0.1
PAPP	7.95	0.24	n.e.	

Therefore, although PAPP displayed appreciable binding affinity, no functional activity was demonstrable. These results suggest that the 5-HT autoreceptor in the guinea-pig frontal cortex at which sumatriptan acts as an agonist, is not identical with the 5-HT_{1D} binding site in this tissue which is identified by the selective ligand [^{125}I]-S-CM-GTNH_2. The reason for this discrepency is under further investigation.

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301P REGIONAL DIFFERENCES IN THE GUINEA-PIG 5-HYDROXYTRYPTAMINE TERMINAL AUTORECEPTOR

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The cortex receives a mixed population of 5-hydroxytryptamine (5-HT) containing nerve fibres, originating from both the dorsal and median raphe nuclei (DRN and MRN respectively). Some cortical areas are preferentially innervated by only one fibre type e.g. the frontal cortex is predominantly innervated from the DRN (Kosofsky and Molliver, 1987). We have previously suggested that multiple autoreceptors may exist in the cortex (Price *et al* 1993). In this study we have used *in vitro* [3H]5-HT release to compare the pharmacology of the terminal autoreceptor in the frontal cortex with that of the whole cortex, to investigate the possibility that fibres arising from different raphe nuclei may contain different populations of autoreceptors.

Guinea-pig brain slices were preloaded with 100nM [3H]5-HT. Release was electrically stimulated for two periods, S1 and S2, both consisting of 2ms biphasic square wave pulses, 20mA in amplitude, at a frequency of 1Hz for 120 pulses. Superfusate samples were collected every four minutes using a Brandel superfusion 2000 apparatus. The autoreceptor agonists 5-HT, 5-carboxamidotryptamine (5-CT) and sumatriptan were superfused during the S2 period. The S2/S1 ratio of fractional release was calculated and results expressed as a percentage of control.

All three agonists produced a concentration dependent inhibition of [3H]5-HT release from both brain regions, with potencies shown in table 1. (Data were analysed through Grafit, using a four parameter logistic fit).

Table 1.

Agonist	Whole Cortex			Frontal Cortex		
	pEC50±s.e.mean	slope	% Emax±s.e.mean	pEC50±s.e.mean	slope	% Emax±s.e.mean
5-HT	7.96±0.16 (n=9)	0.58	83%±6%	7.72±0.10 (n=3)	2.64	53%±6% **
5-CT	8.42±0.14 (n=6)	0.68	98%±1%	8.24±0.18 (n=3)	0.70	86%±7%
Sumatriptan	6.95±0.20 (n=5)	0.98	66%±10%	6.42±0.25 (n=3)	0.72	72%±9%

The maximum inhibitory response varied between agonists. In the case of 5-HT the Emax was significantly lower in the frontal cortex (**$p<0.003$, unpaired t-test), suggesting that a component of the inhibition was absent from the fibres arising from the DRN. In addition the whole cortex 5-HT inhibition curve was very shallow. This combined data is further evidence in support of more than one subtype of 5-HT terminal autoreceptor in the guinea-pig cortex.

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302P CHARACTERIZATION OF THE DISTRIBUTION OF 5-HT$_4$ RECEPTORS IN HUMAN BRAIN USING [^{3}H]GR113808

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GR113808 is a selective antagonist with high affinity for the 5-HT$_4$ receptor (Grossman et al., 1993). This receptor is present in both the periphery and the CNS, with a potential involvement in cardiovascular, gastrointestinal and central neuronal function (Bockaert et al., 1992). In the present study, [^{3}H]GR113808 has been used to determine the regional distribution and pharmacological specificity of 5-HT$_4$ receptors in post-mortem human brain tissue.

The binding assay used tissue homogenized in Tris HCl (25 mM) containing 0.5 mM EDTA at pH 7.4, followed by two cycles of centrifugation at 48,000g and resuspension. The final homogenate was incubated in buffer at a 400-fold dilution with radioligand and displacing agents as appropriate for 30min at room temperature (21 ± 1°C) in triplicates. Non-specific binding was defined in the presence of 0.1 mM tropisetron. The incubation was terminated by rapid filtration and washing with ice-cold buffer. The regional distribution and pharmacological specificity in putamen (using 0.05 nM [^{3}H]GR113808) are summarized in Table 1A and 1B.

Table 1 Regional distribution (A) and pharmacological specificity (B) of [^{3}H]GR113808 binding in human brain

A.

Brain Region	Bmax (fmol.mg tissue^{-1})	Kd (nM)
Lateral pallidum	14.74 ± 1.86	0.122 ± 0.018
Putamen	9.16 ± 1.86	0.073 ± 0.010
Caudate nucleus	8.71 ± 1.49	0.068 ± 0.011
Temporal cortex	2.63 ± 0.56	0.069 ± 0.018
Hippocampus	2.39 ± 0.84	0.055 ± 0.025
Amygdala	2.33 ± 1.12	0.057 ± 0.025
Frontal cortex	1.74 ± 0.50	0.053 ± 0.023

B.

Compound	pKi	nH
GR113808	10.05 ± 0.04	1.02 ± 0.12
SDZ205-557	8.65 ± 0.08	0.96 ± 0.20
DAU 6285	7.94 ± 0.04	0.97 ± 0.06
BIMU 1	7.81 ± 0.06	0.96 ± 0.09
Tropisetron	7.39 ± 0.23	1.08 ± 0.03
5-HT	7.32 ± 1.00	0.95 ± 0.10
R-zacopride	5.82 ± 0.04	1.01 ± 0.12

Values were expressed as mean ± S.D. from 3 brains. Saturation studies used 7 ligand concentrations over the range of 6.25 µM - 0.4 nM.

The displacement studies in the putamen indicated a pharmacological specificity consistent with a 5-HT$_4$ receptor. Similar results were obtained using cerebral cortical tissue. The regional distribution of 5-HT$_4$ receptors in human brain (Table 1), showing low levels in the cerebellar cortex but high levels in the striatum, was consistent with Waeber et al. (1993). There was no evidence of heterogeneity of 5-HT$_4$ receptors in human brain as indicated by unity Hill slopes in the displacement studies. However, the pallidum did exhibit an affinity for [^{3}H]GR113808 two-fold lower than the other regions. In conclusion, these studies with [^{3}H]GR113808 have confirmed the presence of 5-HT$_4$ receptors in the human brain in a regional specific manner.

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303P EFFECT OF SB 200646A, A 5-HT_{2C} RECEPTOR ANTAGONIST, ON TWO CONFLICT MODELS OF ANXIETY

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SB 200646A has 50-fold selectivity for the 5-HT_{2C} and 5-HT_{2B} over the 5-HT_{2A} receptor and at least 80-fold selectivity over all other receptors tested (Forbes et al., 1993). We have previously shown that SB 200646A had anxiolytic-like effects in the rat social interaction test (Kennett et al., 1993a). Its activity in two other anxiety models, with different aversive and motivational bases, are now presented.

Male CFY (400-500 g) rats, fed a restricted diet, were trained to press a lever for a food reward, and to associate a light cue with both a high level of reward [5 presses per reward (FR5)] and a contingent footshock (pulse width 15 msec at intervals of 200 msec for 1 second). This was individually titrated up to 1 mA to give 2-7 reinforcements in total in 5 x 3 min FR5 periods. The 5 FR5 periods were alternated with 5 x 3 min unpunished periods where reward was given at variable intervals averaging 30 secs (VI30) (see Kennett et al., 1993b). In a second study male and female marmosets, (bred in house, 400-500 g) on a normal diet, were trained to lever-press for a banana milk shake reward. Tests consisted of 4 x 2 min unsuppressed periods, alternated by 4 x 2 min suppressed periods where responding was on a fixed ratio titrated for individual animals dependent on performance. The suppressed periods were associated with a light cue and an air puff to the face, which the marmosets find aversive, via the reward dispenser (Piper, 1988).

SB 200646A selectively increased punished responding (mean % change $\pm$s.e.m. of lever-presses from score on two preceding days) in both the rat and marmoset tests 1 h pretest (rat; 5 mg/kg p.o., +82.0 $\pm$30.0 $p<0.01$, 20 mg/kg p.o.+81 $\pm$ 36.8, marmoset; 20 mg/kg p.o., +133.5 $\pm$64.4, $p<0.05$ by log transformation and 2-way ANOVA) with no increase in unpunished responding.

Thus, SB 200646A has anxiolytic properties in both conflict models consistent with its action in the rat social interaction test (Kennett et al., 1993b) and with the hypothesis that 5-HT_{2C} receptor stimulation is anxiogenic (see Kennett et al., 1993b).

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304P A PHARMACOLOGICAL STUDY OF THE mCPP DISCRIMINATIVE STIMULUS

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1-(3-Chlorophenyl)piperazine (mCPP) has been used as an agonist for the 5-HT_{2C} receptor (formally 5-HT_{1C}, Kennett, 1993). Since this compound has also been shown to have discriminative stimulus properties (Winter and Rabin, 1993), it was the purpose of the present study to determine whether its cue is mediated by the 5-HT_{2C} receptor.

Using a two lever FR10 operant drug discrimination paradigm, male Sprague Dawley rats achieved correct discrimination of mCPP (1 mg/kg i.p., 15 min pretreatment) from saline in 10 consecutive 15 min training sessions. Generalisation experiments of 2 min duration with reinforcement available on both levers were then initiated. Increasing cumulative doses of test compounds (mCPP, fenfluramine, MK 212, RU 24969, MDL 72222) were given i.p. 30 min before the test at intervals of 2 hours until mCPP-appropriate responding was seen in at least 80% of the rats tested or until the total response rate was significantly reduced. For antagonism studies, compounds (methiothepin 1 mg/kg i.p., mesulergine 2 mg/kg i.p., pindolol 5 mg/kg i.p. and MDL 72222 3 mg/kg s.c.) were administered 30 min before mCPP 1 mg/kg i.p..

mCPP induced dose-dependently drug lever selection (ED_{50} = 0.48 mg/kg). The 5-HT releaser, fenfluramine (3 mg/kg i.p.), substituted to the mCPP cue, suggesting the involvement of post-synaptic 5-HT receptors. The 5-HT_{2C} agonist, MK 212 (1 mg/kg i.p.) and the 5-$HT_{1A/1B}$ agonist, RU 24969 (1 mg/kg i.p.) generalised to the mCPP cue and reduced the response rate (92 % and 24 % respectively). However, the 5-$HT_{2A/2C}$ antagonist mesulergine, the 5-$HT_{1/2}$ antagonist, methiothepin and the 5-$HT_{1A/1B}$ antagonist pindolol failed to inhibit the mCPP cue although a decrease in the response rate was observed in each case (52 %, 43 % and 57 % respectively). Since mCPP has also been shown to be a 5-HT_3 ligand (Robertson et al., 1992), generalisation (up to 10 mg/kg i.p.) and mCPP antagonism were attempted with MDL 72222 which was ineffective in both cases.

Therefore, these data suggest that although mCPP induces a discriminative stimulus via a post-synaptic 5-HT receptor, it does not appear to be mediated by any of the 5-HT_1, 5-HT_2 or 5-HT_3 receptors in view of the failure of their antagonists to abolish the mCPP cue.

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305P ACCELERATED LEARNING OF A TEMPORAL DISCRIMINATION FOLLOWING DESTRUCTION OF THE ASCENDING 5-HYDROXYTRYPTAMINERGIC PATHWAYS

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The ascending 5-hydroxytryptaminergic (5HTergic) pathways are believed to play a complex role in discrimination learning (McEntee & Crook, 1991). Recently we found that the acquisition of temporal differentiation of responding under a schedule of reinforcement specifying a minimum inter-response time was impaired following destruction of these pathways (Wogar *et al.*, 1992). In the present experiment we examined the effect of the same lesion on the acquisition of a temporal discrimination using an interval bisection task (Morrissey *et al.*, 1993).

Under halothane anaesthesia, 16 rats received injections of 5,7-dihydroxytryptamine (4 μg base in 2 μl vehicle) into the dorsal and median raphe nuclei; 10 rats received sham lesions (for method, see Wogar *et al.*, 1992). The rats were then trained to press levers in conventional operant conditioning chambers using a sucrose reinforcer (50 μl, 0.6 M). In Phase I (40 days), each daily 35-minute session consisted of 100 discrete trials in which the rats were required to press lever A following a 200-ms presentation of a light stimulus, and lever B following an 800-ms presentation of the same stimulus. In Phase II (30 days), 10 'probe' trials (without reinforcement) were introduced into each session, in which the light was presented for intermediate durations (260, 320, 400, 510, 640 ms; two trials in each case). After completion of the experiment, the rats were sacrificed and their brains dissected for determination of the concentrations of 5HT, 5-hydroxyindoleacetic acid (5HIAA), noradrenaline and dopamine by high-performance liquid chromatography with electrochemical detection (for method, see Wogar *et al.*, 1992).

During Phase I both groups showed gradual acquisition of accurate discrimination, attaining 80-90% correct choices within 40 sessions. Analysis of variance revealed that the lesioned group learned the discrimination significantly faster than the control group (group: $F[1,25]=4.2$, $0.1>P>0.05$; sessions: $F[39,975]=67.9$; $P<0.001$; interaction: $F[39,975]=1.9$, $P<0.01$), the between-group difference being statistically significant ($P<0.05$) between sessions 17 and 34. During Phase II the bisection point (duration corresponding to 50% choice of lever B, ± s.e.mean) did not differ significantly between the lesioned (435 ± 13 ms) and control (463 ± 18 ms) groups ($P>0.1$). However discriminative accuracy as defined by the Weber fraction (Morrissey *et al.*, 1993) was superior in the lesioned group (0.35 ± 0.03), compared to the control group (0.55 ± 0.09) ($t[24]=2.2$, $P<0.05$). The levels of 5HT and 5HIAA in the parietal cortex, hippocampus, amygdala and nucleus accumbens of the lesioned group were less than 20% of those of the control group ($P<0.001$), but the levels of noradrenaline and dopamine did not differ significantly between the groups.

The results support the suggestion that the 5HTergic pathways are involved in discrimination learning. However, the facilitatory effect of the lesion on the acquisition of temporal discrimination seen here contrasts with the deleterious effect of the lesion on the acquisition of temporal differentiation of responding (Wogar *et al.*, 1992). The results are consistent with models of timing that posit different mechanisms underlying the temporal regulation of an animal's own behaviour and its ability to discriminate the durations of external stimuli (see Morrissey *et al.*, 1993).

This work was supported by the Medical Research Council.

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306P *EX VIVO* BINDING OF 5-HT_3 ANTAGONISTS TO 5-HT_3 RECEPTORS IN THE RAT ENTORHINAL CORTEX AND COLON

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5-HT_3 receptors can be detected in rat tissues by radioligand binding using ^{3}H-GR65630 (Kilpatrick et al., 1987). Using a modification of this method, we have developed an *ex vivo* binding assay for assessing the CNS penetration of 5-HT_3 antagonists by measuring the competition for ^{3}H-GR65630 by drug that has accumulated in the brain or colon.

Male RH rats (150-200 g) were dosed with 5-HT_3 antagonist or vehicle (n=4). After 5 min (i.v.) or 1 h (s.c.) rats were killed with an overdose of pentobarbitone (100 mg i.p.). Rats were then perfused transcardially with saline (30 ml at 4°C) and the entorhinal cortices and colon dissected and frozen. For the binding assay, tissues were thawed and homogenized individually in HEPES buffer (50 mM; pH 7.4; 20 vol). The colon homogenate was then filtered through a porous cloth. Specific binding was determined using the methodology of Kilpatrick et al. (1987) except that the assay contained: 300 μl of tissue homogenate, 50 μl of ^{3}H-GR65630 (specific activity 84.5 Ci mmol^{-1}; 0.32 nM) and 50 μl of HEPES or 30 μM metoclopramide or drug. Affinities (pKi values) were estimated in tissues from saline-perfused rats. The dose of antagonist that decreased specific binding by 50% (ID_{50} values with 95% confidence limits) was determined from dose-effect curves.

Table 1. Comparison of ID_{50} values from *ex vivo* binding with pKi values of 5-HT_3 antagonists in the rat entorhinal cortex and colon

	Entorhinal cortex ID_{50} mg kg^{-1}	Colon ID_{50} mg kg^{-1}	ID_{50} cortex / ID_{50} colon	pKi entorhinal cortex	pKi colon	log D_7*
Ondansetron	0.20 (0.13-0.32) i.v.	0.034 (0.013-0.094) i.v.	5.9	8.7	9.0	1.30
	0.75 (0.48-1.37) s.c.	0.16 (0.03-9.02) s.c.	4.7			
MDL72222	0.44 (0.24-0.99) i.v.	0.55 (0.39-0.82) i.v.	0.8	7.3	7.6	2.01
	0.34 (0.19-0.61) s.c.	0.33 (0.20-0.56) s.c.	1.0			
GR75244.	11 (3.9-266) i.v.	0.9 (0.07-2.40) i.v.	12.2	8.2	8.3	-0.39

* Log D_7 = measured partition coefficient between octanol and water at pH 7.0 GR75244 = N-methyl quaternary salt of ondansetron

The affinity of the antagonists generally correlates with their ID_{50} values in the colon, but not in the entorhinal cortex (Table 1). The discrepancies in the cortex ID_{50} values are not due to a difference in the affinities of the antagonists for the tissues. However, the ratio of potency in cortex/colon does correlate with lipophilicity of the antagonists. We conclude that the CNS penetration of 5-HT_3 antagonists can be assayed using *ex vivo* binding.

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307P MODULATION OF CALCITONIN GENE-RELATED PEPTIDE RELEASE FROM RAT SPINAL CORD SLICES

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Within the spinal cord calcitonin gene-related peptide (CGRP) is located in primary afferent nerve terminals in the dorsal horn, with lower levels also in ventral horn motoneurones. Serotonergic neurones make close contact with CGRP containing neurones in both these spinal regions, therefore the present study examined the effect of 5-HT$_3$ receptor activation on release of endogenous CGRP-like immunoreactivity (LI) from both dorsal and ventral spinal cord slices, using a fixed volume incubation technique.

Male wistar rats (320-400g) were decapitated and dorsal (third) and ventral (two-thirds) portions of the thoraco-lumbar spinal cord were dissected into bilateral halves, sliced, washed and resuspended in Krebs (1ml) containing bacitracin (1.8 U ml^{-1}) and aprotinin (10 KIU ml^{-1}) as described previously (Fone *et al.*, 1991). Slices were gased (95%O$_2$/5%CO$_2$) and pre-incubated with i). 2-methyl-5-hydroxytryptamine (2-Me-5-HT, 10^{-7} to 10^{-5}M) either alone or after ondansetron (10^{-8}M, -2 min) or Krebs in a shaking water bath (37°C, 5 min) before adding K+ (50mM), or incubated with ii). K+ (25 to 100mM) or iii). capsiacin (10μM) and shaken for a further 20 min. Slices were centrifuged (2300g, 2°C for 5 min) to separate supernatant and tissue pellet and determine rat αCGRP-LI in each by radioimmunoassay (see Fone, 1992). Results (given as mean ± s.e.mean, n = 5-12 in each group) were analysed by Student's t-test.

Potassium (25, 50 and 100mM) produced a dose-related increase (263 ± 43 not significant, 303 ± 66 $P<0.05$ and 404 ± 38 $P<0.01$, respectively) in basal (149 ± 38 fmol mg protein^{-1}, which was 12 ± 1% of tissue content) CGRP-LI release from dorsal slices, which was significantly attenuated (to 170 ± 27, $P<0.05$ from 50mM K$^+$ controls) by incubation in a calcium free medium containing EDTA (1mM). Basal CGRP-LI release from ventral slices (39 ± 6 fmol mg protein^{-1}, n=21, being 10 ± 4% of tissue content) tended to be elevated by potassium (by 41, 53 and 126% above control with 25, 50 and 100 mM, respectively) but this failed to reach significance and was not significantly reduced by incubation in a calcium free medium. Capsiacin significantly elevated CGRP-LI release from dorsal slices (by 163%, $P<0.05$). Pre-incubation with the 5-HT$_3$ receptor agonist 2-Me-5-HT (10^{-7}, 10^{-6} and 10^{-5}M) attenuated potassium (50mM) evoked CGRP-LI release from dorsal slices in a dose-related manner (being unaltered, reduced by 32%; not significant and 38%; $P<0.01$ from 50mM K+ controls, respectively). Pre-incubation with ondansetron alone did not affect potassium-evoked CGRP-LI release but ondansetron reversed the inhibition produced by addition of 2-Me-5-HT to dorsal slices.

Potassium-induced CGRP-LI release from dorsal cord slices was calcium dependent and release was also evoked by capsiacin, consistent with the preposal that the CGRP-LI is principally from primary afferent terminals in this region. The attenuation of potassium-induced CGRP-LI release from dorsal slices evoked by 2-Me-5-HT was prevented by ondansetron suggesting that activation of 5-HT$_3$ receptors, which are located on primary afferent terminals (Hamon *et al.*, 1989), attenuates CGRP release. The low levels of CGRP-LI released from ventral slices may come from motoneurone terminals or dendrites but was too variable to be investigated further.

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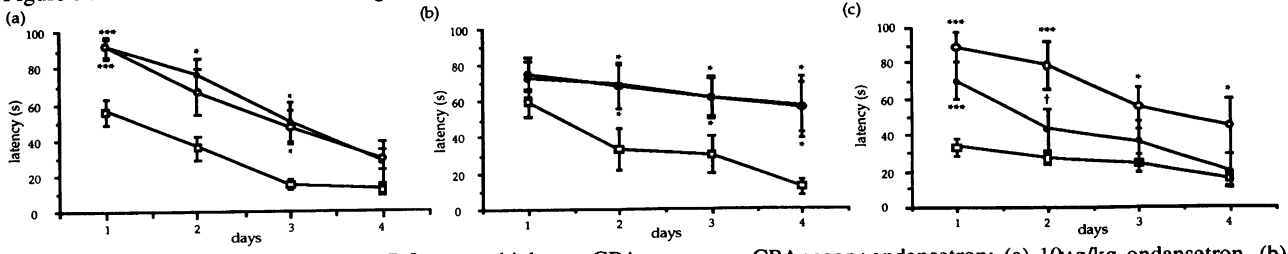
308P EFFECT OF ONDANSETRON ON A WATER MAZE ACQUISITION DEFICIT PRODUCED BY COMBINED pCPA AND SCOPOLAMINE TREATMENT

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A previous study (Harder *et al*, 1993) has demonstrated a consistent deficit in spatial learning in the rat in a water maze with combined p-chlorophenylalanine (pCPA) and scopolamine treatment. Current studies examined the effect of the putative cognitive enhancer, ondansetron, (Costall & Naylor, 1991) on the impairment produced by the combined treatment.

Male Lister Hooded rats (250-450g) were trained for 4 trials per day over 4 days in a water maze following 3 days of pCPA treatment (100mg/kg ip daily). Ondansetron (10μg/kg, 100μg/kg or 1mg/kg, ip) was administered 40 min before maze exposure, followed 20 min later by scopolamine (scop; 0.25mg/kg, ip) as a 20 min pretreatment. The time taken to locate the submerged island (latency) was used as the measure of learning ability. Scopolamine alone failed to modify behavioural responding.

Figure 1 : Latencies to find the submerged island (s).



Data are presented as mean ± sem, n=7-8. - - vehicle - -pCPA+scop - - pCPA+scop+ondansetron; (a) 10μg/kg ondansetron, (b) 100μg/kg ondansetron, (c) 1mg/kg ondansetron. *$p<0.05$, ***$p<0.005$ compared to vehicle control group; †$p<0.05$ compared to pCPA + scopolamine treated group; one factor ANOVA followed by Dunnett's t test.

In all 3 experiments, pCPA and scopolamine treatment produced a significant impairment in acquisition of the water maze task, as shown by the increased latency relative to vehicle controls. Ondansetron (10μg/kg and 100μg/kg) did not alleviate this impairment; no significant difference between pCPA/scopolamine treatment and pCPA/scopolamine/ondansetron treatment. Treatment with ondansetron (1mg/kg) did cause some alleviation of the deficit, with pCPA/scopolamine/ondansetron treated animals showing similar latencies to vehicle controls on days 2, 3 and 4, and finding the island with significantly reduced latencies than pCPA/scopolamine treated rats on day 2.

The combination of 5-HT depletion and muscarinic antagonist treatment has been suggested as a model for Alzheimer's disease (Richter-Levin & Segal, 1989). This study provides evidence of limited cognitive enhancing properties of ondansetron in a potential paradigm for dementia disorders.

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309P EFFECTS OF ACUTE AND CHRONIC TREATMENTS WITH TIANEPTINE AND PAROXETINE ON 5-HTP-EVOKED CHANGES IN 5-HT CONTENT OF RAT HIPPOCAMPAL DIALYSATES

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Tianeptine is a novel tricyclic antidepressant which differs from other tricyclics because it increases neuronal 5-hydroxytryptamine (5-HT) uptake (Mennini et al., 1987). Whereas acute administration of classical tricyclic antidepressants and non-tricyclic 5-HT uptake inhibitors potentiated 5-HT dependent behaviours evoked by L-tryptophan or by 5-hydroxytryptophan (5-HTP) in rats and potentiated spinal reflex activity, tianeptine attenuated these behaviours (Curzon et al., 1990; Koshikawa et al., 1991). The purpose of the present study was to determine whether these differences persist after chronic administration.

Male Wistar rats (180-220 g) were treated with tianeptine (10 mg kg^{-1}), paroxetine (10 mg kg^{-1}) or saline (0.1 ml kg^{-1}) daily and challenged with L-5-HTP (150 mg kg^{-1} 40 min after a single injection of antidepressant or 40 min and 24 h after 4 weeks chronic administration. One week before experiment, a guide cannula had been implanted (A +4.0 mm, L 5.0 mm, V 6.3 mm above the intra-aural line, Paxinos and Watson, 1986) to allow the insertion of a dialysis probe into the dorsal hippocampus. The tubing was perfused with modified Ringer solution (2.0 µl min^{-1}; pH 6.0). Perfusate 5-HT content was determined every 20 min by an HPLC system (EICOM, Kyoto) after separation on an Eicompac CA-5ODS column (detection limit approx. 1 pg per sample). Drugs were not injected until the concentration of 5-HT in the dialysates had stabilized (at least 3 h after probe insertion). The mean of the last three samples before the drug injection being used as the control baseline value (100%). The rats were given carbidopa (25 mg kg^{-1}) 40 min before 5-HTP.

5-HTP injected into acute and chronic saline control rats produced an approximately 3 fold increase in the 5-HT content of hippocampal dialysates over the ensuing 160 min with no significant differences between the groups (n=6 for each group). When given 40 min after a single injection of paroxetine, 5-HTP increased the dialysate 5-HT content over this period of time by 577% (n=6) whereas after tianeptine 5-HTP content was reduced to 55% (n=6) of control ($P<0.0001$). These percentages were not significantly altered when 5-HTP was given 40 min after the last of 4 weeks daily injections of paroxetine and tianeptine (450% and 59% respectively, $P>0.4$) demonstrating that the effects of paroxetine and tianeptine on uptake processes did not change with chronic treatment. When given 24 h after chronic treatment with either paroxetine or tianeptine, 5-HTP tended to increase the amount of 5-HT recovered from hippocampal dialysates above that seen in the chronic saline control group but the increases, 141% and 133% respectively, were not significant ($P>0.1$). Therefore tianeptine given chronically continues to facilitate 5-HT uptake, an action difficult to accommodate within existing serotonergic hypotheses of antidepressant action.

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310P HYPOPHAGIC EFFECT OF FLUOXETINE IN RATS IS NOT MEDIATED BY INHIBITION OF 5-HT REUPTAKE OR AN AGONIST ACTION AT 5-HT_{2C} RECEPTORS

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Fluoxetine may cause hypophagia in rats independently of 5-HT reuptake (Grignaschi and Samanin 1992), perhaps via its affinity for the 5-HT_{2C} receptor (Wong et al., 1991), stimulation of which is hypophagic. The effect of fluoxetine on feeding in rats given the 5-HT synthesis inhibitor, p-chlorophenylalanine (PCPA), and on 5-HT_{2C} receptor function in the pig choroid plexus (CP) was studied.

Male CD rats (250-300 g) were pretreated with PCPA methyl ester, (150 mg/kg i.p.) or vehicle (0.9% saline) daily for 3 days. After the last treatment the animals were housed singly and food-deprived for 23 hours prior to testing. On the test day animals from each group (n=17-20) were given fluoxetine (20 or 30 mg/kg p.o.) or vehicle (1% methyl cellulose). One hour later food was restored and the amount eaten by each animal over 4 hours recorded. Results (mean±s.e.m.) were analysed by Newman Keuls test after a significant 2 way ANOVA. Inhibition of [^{3}H]-mesulergine binding to pig CP by fluoxetine was determined as in Pazos et al., (1984) and its effect on 5-HT-stimulated phosphoinositide (PI) hydrolysis in piglet CP as in Brown et al., (1991).

In vehicle pretreated animals fluoxetine, at 20 and 30 mg/kg, significantly reduced ($p<0.01$) food intake from 7.77 ± 0.30g to 4.04 ± 0.58g and 5.52 ± 0.43g, respectively. PCPA pretreatment alone, did not alter feeding on the test day (7.36 ± 0.44g), nor did it inhibit the effect of fluoxetine, which at 20 and 30 mg/kg, reduced ($p<0.01$) food intake to 3.87 ± 0.44g and 3.37 ± 0.38g, respectively. In binding studies fluoxetine inhibited specific [^{3}H]-mesulergine binding, in the pig CP, with a pK_I of 6.45 ± 0.09 and a slope factor of 1.06 ± 0.14 (n=4). Furthermore in slices of piglet CP, 5-HT caused a circa 10 fold stimulation in PI hydrolysis with an EC_{50} of 45nM ± 3 (n=3) and fluoxetine (1-33 µM) caused a rightward shift in the dose-response curve produced by 5-HT with no effect on the maximal response, giving a pK_B of 5.94 ± 0.09, n=3 experiments.

PCPA did not inhibit fluoxetine-induced hypophagia, which is therefore unlikely to be mediated by increased synaptic cleft 5-HT. In addition, since fluoxetine acted as an antagonist at the 5-HT_{2C} receptor, its hypophagic effect in rats is also unlikely to be mediated by stimulation of the 5-HT_{2C} receptor. These results thus suggest the involvement of an additional, unknown mechanism.

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311P *IN VIVO* INACTIVATION OF RAT CORTICAL 5-HT$_{2A}$ RECEPTORS BY EEDQ: PROTECTIVE EFFECTS OF 5-HT AGONISTS AND ANTAGONISTS

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N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) is a highly reactive molecule which irreversibly inactivates rat brain 5-HT2A receptors in vitro and in vivo (Battaglia et al., 1986). The mechanism involves reaction with peptide carboxyl groups situated at or near the active binding site (Chang et al., 1970). Previous studies have shown that dopamine D1 and D2 and alpha2-adrenergic agonists and antagonists protect their respective receptors from EEDQ inactivation (Saller et al., 1989; Pilc et al., 1992). The aim of this study was to quantitate the inactivation of 5-HT2A receptors after peripheral administration of EEDQ and to determine the protective effects of a variety of ligands for the 5-HT2A receptor in rat brain frontal cortex.

Male CD rats (120-140g; Charles River) received drugs or saline i.p. 30min prior to administration of EEDQ (6mg/kg ip) or vehicle. Frontal cortices were removed 24h after the final treatment and frozen at -20°C until assay. Cortical membranes (5mg wet weight tissue/ tube) were incubated with [3H]ketanserin at 8 concentrations (0.05-4.8nM) for 15min at 37°C. Non-specific binding was defined by methysergide (5μM). Binding parameters (Kd and Bmax) were determined using weighted non-linear least-squares curve fitting (LIGAND). Comparisons between groups were made using two-way ANOVA for two independent variables.

Table 1 EEDQ-induced decreases in 5-HT2A receptor number: effect of pretreatment with 5-HT receptor ligands

TREATMENT	SALINE/VEHICLE	DRUG/VEHICLE	% change	SALINE/EEDQ	DRUG/EEDQ	% protection
Ritanserin (1)	365 ± 15	288 ± 11 ††	-21	123 ± 5 ††	250 ± 12 **	+77
Ketanserin (1)	434 ± 10	369 ± 12 ††	-15	117 ± 7 ††	283 ± 14 **	+66
Metergoline (3)	440 ± 18	351 ± 11 ††	-20	141 ± 6 ††	294 ± 18 **	+73
DOI (10)	415 ± 14	234 ± 17 ††	-44	112 ± 3 ††	172 ± 8 **	+49
Spiroxatrine (1)	488 ± 12	468 ± 13	-4	193 ± 23	160 ± 4	-12
8-OH-DPAT (1)	364 ± 9	381 ± 12	+5	154 ± 14	136 ± 8	-7
RU 24969 (5)	364 ± 9	397 ± 18	+9	154 ± 14	117 ± 5	-15

Bmax (fmol/mg protein) ± s.e.mean (n=9-10). Dose mg/kg in parentheses. **p<0.01 vs saline/EEDQ, ††p<0.01 vs saline/vehicle

[3H]Ketanserin binding was of high affinity and fitted to a single site binding model in all cases. EEDQ significantly reduced the number of 5-HT2A receptors by 57%-73% without altering the Kd. A single injection of agonist (DOI, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) or antagonists (ritanserin; ketanserin; metergoline) with high affinity for 5-HT2A receptors also significantly decreased these receptors. However, these compounds protected 5-HT2A receptors against EEDQ inactivation. By contrast, drugs with affinities for 5-HT1A or 5-HT1B receptors (spiroxatrine; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin; RU 24969, 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole) had no effect on 5-HT2A receptor number and did not protect against EEDQ. The results, therefore, show that 5-HT2A ligands not only decrease these receptors, but also protect them against EEDQ. Furthermore, as protection was afforded by 5-HT2A agonists and antagonists, it appears that EEDQ does not differentiate between affinity states of the 5-HT2A receptor.

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312P BLOCKADE OF RAT BRAIN MONOAMINE UPTAKE SITES BY ANTIDEPRESSANT DRUGS AND SUBSEQUENT EFFECTS ON CIRCADIAN LOCOMOTOR ACTIVITY RHYTHMS

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The effect of antidepressants on the free-running period of circadian rhythms is controversial, (Wollnik, 1992). We have studied both the changes in locomotor activity rhythms and the levels of brain monoamine uptake in rats treated chronically with antidepressant drugs. Two of these compounds exert their effects via inhibition of monoamine uptake and one, an MAO inhibitor, is not expected to have an effect on monoamine uptake. Male Wistar rats (300 ± 5g Expt. 1; 325 ± 5g Expt. 2) were housed singly in cages equipped with running wheels (35 cm diam.), under constant dark (Expt. 1) or constant dim red (Expt. 2) lighting conditions, ambient temp. 25°C. After four weeks wheel-running, activity patterns had stabilised and animals were administered paroxetine, clorgyline, imipramine or vehicle either via miniosmotic pumps implanted s.c. (Expt. 1) or in their drinking water (Expt. 2). Activity rhythms were monitored throughout drug treatment and the change in circadian period (tau) calculated as the difference between pre- and post- drug values. Rats were then sacrificed and the degree of inhibition of both tritiated serotonin ([^{3}H]5-HT) and tritiated noradrenaline ([^{3}H]NA) uptake was assessed *ex vivo* (Thomas, *et al.*, 1987).

Treatment	Expt. 1				Expt. 2			
	change in tau (h)	[^{3}H]5-HT uptake*	[^{3}H]NA uptake*	concentration mg/kg/day	change in tau (h)	[^{3}H]5-HT uptake*	[^{3}H]NA uptake*	concentration mg/kg/day
vehicle	0.00±0.03	-	-	-	0.07±0.03	-	-	-
paroxetine	0.07±0.04	30.66±13.9	2.26±1.46	0.5	0.16±0.02	67.81± 2.65(4)	11.29± 3.42 (6)	10
clorgyline	0.09 ± 0.06	12.33 ± 6.28	17.4 ± 2.79	1	0.09 ± 0.02	10.28 ± 3.78 (6)	40.74 ± 10.02 (6)	1
imipramine	-0.03±0.04	16.1 ± 10.2	43.34 ± 8.77	10	0.08 ± 0.03	- (6)	30.19 ± 8.29 (6)	10

*mean % inhibition of uptake compared to control animals ± SEM (n=7 unless stated).

Biochemical determinations confirmed the expected pattern of monoamine uptake blockade for paroxetine and imipramine, with the MAO-type A inhibitor clorgyline being unexpectedly potent at blocking NA uptake. A greater inhibition of [^{3}H]5-HT uptake was attained following paroxetine administration in the drinking water because of the higher concentration which could be administered via this route. None of the antidepressant treatments had a significant effect on tau in either experiment indicating that selective inhibition of uptake of either 5-HT (paroxetine) or NA(imipramine) has no effect on rat circadian period.

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313P EFFECTS OF IMIPRAMINE, MIANSERIN AND PHENELZINE ON SOCIAL BEHAVIOUR IN MICE

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Chronic administration of antidepressant agents can increase aggressive behaviour of isolated resident rats (Mitchell et al. 1991), an effect which may relate to the onset of antidepressant efficacy. It recently has been shown that many anxiolytic agents possess antidepressant efficacy and that antidepressants are effective in the treatment of several anxiety states (Lader, 1989). The present experiments examine effects of the antidepressant compounds, mianserin, phenelzine and imipramine, on behaviour of mice in a social interaction test previously employed for the assessment of anxiolytic potential (Gao & Cutler, 1992).

Compounds were administered in drinking fluid for 2 weeks to adult male pair-housed CD1 mice weighing 35-45 g. [n = 16/group] (Imipramine hydrochloride [Sigma] at 21.5 [LD] and 106.5 [HD] mg/l [5 and 20 mg/kg daily]; mianserin hydrochloride [Sigma] at 18.0 [LD] and 57.6 [HD] mg/l [3.6 and 14.4 mg/kg daily] and phenelzine sulphate [Sigma] at 11.5 [LD] and 84.2 [HD} mg/l [2.3 and 18.7 mg/kg daily]. Control mice (n = 20) received tap water to drink. Behaviour shown by each of the mice during social interactions with an untreated partner in a neutral cage was recorded for a 5 min period, using the ethological procedures described by Gao & Cutler (1992).

Time spent by the mice in social investigation was significantly increased by imipramine at both dose levels (Control 26.2±4.0; Treated, LD 46.1±6.4*, *P<0.05; HD 43.2±3.8*, *P<0.05), and by mianserin (Control 25.3±3.0; Treated, LD 49.8±9.2*, *P<0.05; HD 30.2±4.2) and phenelzine (Control 26.2±4.0; Treated, LD 50.4±6.8*, *P<0.05; HD 39.2±5.7) at the smaller of the two dose levels tested. Time spent in aggression was significantly increased by phenelzine at the highest dose level (Control 6.9±3.5; Treated, LD 15.6±4.9; HD 22.0±5.8*, *P<0.05), but not by imipramine or mianserin.

It can be seen that enhancement of aggressive behaviour was not consistently produced by antidepressant compounds in this test situation, but that each of the compounds increased social investigation. The enhancement of social investigation resembles effects previously observed in mice that had been treated with anxiolytic compounds (Gao & Cutler, 1992), and provides further support for the proposal of commonalities between the behavioural actions of these two classes of drug.

Results expressed as mean duration (s.) ± S.E.M. LD = low dose; HD = high dose. Statistics; Mann-Whitney U and Kruskal-Wallis Tests.

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314P PHARMACOLOGICAL CHARACTERIZATION OF THE CLONED HUMAN 5-HT TRANSPORTER

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The sodium-coupled 5-hydroxytryptamine (5-HT) transport system (or 5-HT transporter) mediates the uptake of 5-HT into a variety of cell-types (e.g. serotonergic neurones, platelets, mast cells and endothelial cells), thereby reducing extracellular concentrations of this biogenic amine. This 5-HT transporter has been isolated using a PCR strategy from rat and very recently from man (Ramamoorthy *et al.*, 1993) and identified as a 630 amino acid polypeptide with twelve putative transmembrane spanning domains. In the present study we have investigated the pharmacological properties of the human 5-HT transporter polypeptide subsequent to its transient expression in a surrogate HeLa cell-line.

The human 5-HT transporter cDNA subcloned in the pRC-CMV expression vector was used to transiently transfect exponentially-growing HeLa cells by the calcium phosphate precipitation technique (Chen & Okayama, 1987). The presence of functional transporter was determined 20-72 h after transfection by [^{3}H]-5-HT uptake assays at 37°C where nonspecific uptake was defined as that remaining in the presence of 10 µM fluoxetine.

The transfected cells exhibited concentration dependent, and saturable specific [^{3}H]-5-HT uptake of relatively high-affinity (K_m = 0.55 ± 0.01 µM; mean ± s.e.m. of 3 experiments). The pharmacological profile of specific [^{3}H]-5-HT uptake into the transfected HeLa cells was investigated. The selective 5-HT uptake inhibitors, indalpine, litoxetine, sertraline and fluoxetine potently inhibited [^{3}H]-5-HT uptake with Hill coefficients close to unity and with K_i values (mean ± s.e.m., n=3) of 2.8 ± 0.1 nM, 6.1 ± 1.1 nM, 7.7 ± 1.8 nM and 42.0 ± 6.6 nM, respectively. In contrast, the selective noradrenaline uptake inhibitor, maprotiline, the selective dopamine uptake inhibitor, GBR12909, and the 5-HT_3 receptor antagonist, ondansetron, had very little effect on [^{3}H]-5-HT uptake, with K_i values of > 10 µM, > 1 µM and > 10 µM, respectively.

These expression experiments indicate that the human 5-HT transporter polypeptide of 630 amino acids confers the full pharmacological characteristics necessary for sodium-coupled 5-HT uptake.

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315P EFFECT OF 5-HT$_3$ RECEPTOR LIGANDS ON *IN VIVO* ACETYLCHOLINE RELEASE FROM THE RAT HIPPOCAMPUS

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5-HT$_3$ receptor ligands have been demonstrated to modulate *in vitro* acetylcholine (Ach) release from rat and human brain tissue (Barnes *et al.*, 1989; Maura *et al.*, 1992). However, recent studies by Johnson *et al.* (1993) reported the lack of modulatory influence of 5-HT$_3$ receptor ligands on K^+ stimulated Ach release in rat entorhinal cortex. The present study aims to investigate whether the 5-HT$_3$ receptor agonist, 1-(m-chloro)-phenylbiguanide (McPBG) or the 5-HT$_3$ receptor antagonist, ondansetron exert any influence on *in vivo* Ach release from the rat hippocampus. Microdialysis probes were implanted into chronically indwelling cannulae located in the right hippocampus of male Hooded Lister rats (375-500g) 16 hours prior to the experiment. Probes were perfused with artificial cerebrospinal fluid containing 2 µM neostigmine at a rate of 2.0 µl min^{-1}. After a 100 min stabilisation period, 20 min samples were collected and immediately analysed for Ach by HPLC-ECD in conjunction with pre-choline oxidase and post-choline oxidase/Ach esterase enzyme reactors similar to the method described by Mark *et al.* (1992). After four basal levels (t=0), McPBG (1, 10 and 100 µM) or ondansetron (10 and 100 µM) were administered centrally via the microdialysis probe for a 4 min (McPBG) or 40 min (ondansetron) period.

McPBG at concentrations of 1, 10 and 100 µM (indicated by arrows) failed to significantly modify basal Ach release (basal levels: 152.2 ± 19.4 pg 40µl^{-1}, n = 6; P>0.05, compared to the basal levels, one-way ANOVA, followed by Duncan's t test) from the hippocampus of the rat (Figure 1). Ondansetron at concentrations of 10 and 100 µM (indicated by horizontal bars) also had no significant effect on the extracellular levels of Ach (basal levels: 148.2 ± 20.6 pg 40µl^{-1}, n = 11).

The results suggests that in the rat, under the present conditions, the *in vivo* release of Ach from the hippocampus is not moderated by the 5-HT$_3$ receptor ligands.

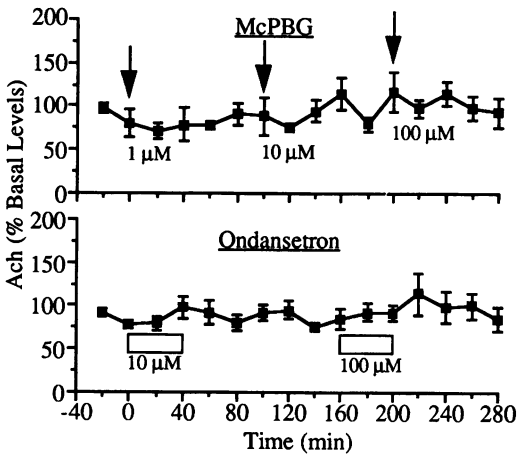


Figure 1. The effect of McPBG or ondansetron on *in vivo* Ach release from rat hippocampus. Data represents the mean ± S.E.M. of 6-11 determinations

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316P 5-HT$_3$ AGONISTS CAN DISTINGUISH CLONED 5-HT$_3$ RECEPTORS FROM NCB20 AND NG108-15 CELL LINES

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The 5-HT$_3$ receptor, which contains an integral cation channel, is a member of the family of ligand-gated ion channels. A subunit for this protein has been cloned, the 5-HT$_3$A subunit, initially from the NCB20 neuroblastoma x chinese hamster cell line and now also from other neuronal cells (Maricq et al., 1989, Hope et al., 1993, Werner et al., 1993). The latter studies have shown the subunit exists in two forms which differ by 6 amino acids containing a potential phosphorylation site in the intracellular loop. We have examined the effects of agonists on homomeric receptors using subunits derived from two clonal cell lines: a long (6 amino acid containing) form from NCB20 cells (NCB-L; Maricq et al., 1991) and a short form from the related cell line NG108-15 (NG-S; Werner et al., 1993).

Full length 5-HT$_3$ DNA sequences from the two cell lines were inserted into the eukaryotic expression vector pRc/CMV and transfected into human embronic kidney 293 cells using calcium phosphate precipitation. Application of 5-HT and the 5-HT$_3$ agonists m-chlorophenylbiguanide (mCPBG) and 2-methyl-5-HT (2-Me-5-HT) induced fast, rapidly-desensitizing inward currents which were reversibly blocked by the 5-HT$_3$ antagonists ondansetron and granisetron at 10nM. EC$_{50}$s for the cloned homomeric receptors compare well to those obtained from NG108-15 cells under identical conditions (Table 1).

Table1: Comparison of agonist EC$_{50}$ values for homomeric and native 5-HT$_3$ receptors (mean ± SE, n ≥ 4; data for NG108-15 from Boess et al., 1991).

EC$_{50}$ (µM)	NG108-15	NG-S	NCB-L
5-HT	4.4 ± 0.3	1.6 ± 0.02	0.9 ± 0.06
mCPBG	3.1 ± 0.3	0.9 ± 0.03	0.2 ± 0.02
2-Me-5-HT	17.7 ± 3.0	13.5 ± 0.78	7.2 ± 0.44

Analysis of agonists effects, however, were able to distinguish between the different receptors. Application of 2-Me-5-HT to NG-S receptors revealed it was a partial agonist, reaching a maximum response which was 56.3 ± 1.1% of the maximum 5-HT-induced response; when applied to NCB-L receptors, however, 2-Me-5-HT was a full agonist (89.2 ± 1.9% of the maximum 5-HT response). Thus we conclude that there are differences between the two homomeric receptors.

We would like to thank E. Kawashima and K. Jones for the NG108-15 clone and D. Julius for the NCB20 clone. SCRL is a Royal Society University Research Fellow

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317P CHARACTERISATION OF A 5-HT_{1B} RECEPTOR ON CHO CELLS: FUNCTIONAL RESPONSES IN THE ABSENCE OF RADIOLIGAND BINDING

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Chinese hamster ovary(CHO) cells have been reported to be devoid of 5-HT receptors and have frequently been used as hosts for the expression of cloned 5-HT receptors. Unexpectedly, we have found that 5-HT induces profound inhibition of forskolin-stimulated cAMP production in these cells and the aim of this study was to classify the 5-HT receptor involved.

cAMP was measured in CHO-K1 cells or the mutant CHO(dhfr-) cell line which were grown to confluency in 12-well plates. After agonist addition cells were stimulated with 1μM forskolin for 10min. Antagonists were pre-incubated for 45min prior to agonist addition. cAMP was extracted in ethanol and measured by scintillation proximity assay. Purified membranes were used for saturation binding experiments and incubated with ^{3}[H]-5-HT or ^{125}[I]-cyanopindolol at 27°C for 30min.

In CHO(dhfr-) cells 5-HT was a potent agonist ($p[A_{50}]$ = 8.07±0.05) and caused 80-100% inhibition of forskolin stimulated cAMP production. A study using several 5-HT_1 receptor agonists revealed the following potencies ($p[A_{50}]$): RU24969 (9.09±0.17) > 5-carboxymidotryptamine (8.86±0.20) > 5-HT (8.07±0.05) > CP93129 (7.74±0.10) > sumatriptan (5.93±0.04), n≥4. All five agonists achieved a similar maximum effect. The 5-$HT_{1A/1B}$ antagonist, (±)cyanopindolol (4-100nM), caused parallel rightward shift of the 5-HT concentration-effect (E/[A]) curve with no change in asymptote. Schild analysis yielded a pK_B estimate of 8.69±0.09 (13df, slope 1.13±0.10). Cyanopindolol actually behaved as a partial agonist with an intrinsic activity of 0.2-0.5 and a $p[A_{50}]$ of 8.55 (n=2). This agonist and antagonist profile is consistent with the presence of a 5-HT_{1B} receptor. 8-OH-DPAT (1μM) and renzapride (3μM) were without effect on forskolin-stimulated cAMP production and ketanserin (0.3μM) did not antagonise the inhibition produced by 5-HT, thus excluding the involvement of 5-HT_{1A}, 5-HT_4, or 5-HT_2 receptors.

We wondered if the expression of a 5-HT_{1B} receptor in our CHO(dhfr-) cells had occurred at the time of the dhfr- mutation. However, this was not the case since RU24969, 5-HT and CP93129 were also potent agonists in CHO-K1 cells: $p[A_{50}]$ 9.03±0.03, 8.34±0.05, 7.69±0.07 respectively (n=3), and cyanopindolol (0.1μM), right-shifted the 5-HT E/[A] curve and yielded a pA_2 estimate of 8.70±0.06 (n=3). Surprisingly, we could not detect any specific binding of ^{3}[H]-5-HT (0.1-200nM), or of the higher affinity and higher specific activity ligand ^{125}[I]-cyanopindolol (0.01-3nM). This was unexpected in view of the high potency of 5-HT and the partial agonist activity of the normally 'silent' competitive antagonist, cyanopindolol. We can only surmise that this high efficacy is due to very efficient receptor- effector coupling of a low density of 5-HT_{1B} receptors.

In, summary, cultured CHO cells express 5-HT_{1B} receptors which are negatively coupled to adenylyl cyclase and this is a simple assay in which to measure functional responses at this receptor. Clearly, the absence of detectable radioligand binding cannot be assumed to mean the absence of receptors capable of eliciting a significant functional response.

318P NEOCORTICAL HYPERAEMIA PRODUCED IN RAT BY ACUTE EXPOSURE TO METHYLENE-DIOXYMETHAMPHETAMINE

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Recent reports have suggested a link between exposure to methylenedioxymethamphetamine (MDMA; "Ecstasy") and the occurrence of intracerebral haemorrhage *(Harries & DeSilva, 1992)*. It is not clear however whether it is MDMA itself, or some contaminant in illicit "Ecstasy" which is responsible for the pathological cerebrovascular disturbance. In this study, we have examined the acute effects of a single exposure to MDMA upon local cerebral blood flow (LCBF) and glucose metabolism (LCGU) in the conscious rat brain. The purpose of the investigation was to determine whether MDMA has the potential to alter cerebrovascular regulation.

Conscious, lightly restrained adult rats *(Sokoloff et al., 1977)* were injected (i.v.) with either MDMA (5mg/kg; n = 12) or saline (0.6ml; n = 12). Equal numbers of treated and control rats were used for the measurement of LCGU (μmol/100g/min) and LCBF (ml/100g/min) using the fully quantitative [^{14}C]-2-deoxyglucose *(Sokoloff et al., 1977)* and [^{14}C]-iodoantipyrine *(Sakurada et al., 1978)* techniques respectively. Tracer concentrations were measured by liquid scintillation analysis of tissue samples dissected from neocortex, hippocampus and striatum, but in a further two animals from each group, the brains were processed for quantitative autoradiographic analysis. Blood pressure was monitored continuously Physiological data were analysed by Scheffe's test and LCGU and LCBF measurements were analysed by Student's t-test, with acceptable levels of significance set at $P < 0.05$.

The injection of MDMA produced transient arterial hypertension in the range 160-190mmHg (cf. 131± 6mmHg preinjection) which was rapid in onset, but lasted no more than 2-3 minutes. There were no significant effects of MDMA upon any other physiological parameter measured (arterial pH, pCO2, O2, plasma glucose, rectal temperature). Despite the intense motor activity in MDMA-treated rats, there were no significant changes in LCGU in any of the brain areas analysed in this study. In contrast, LCBF in frontal (174 ± 20) and parietal cortices (199 ± 30) were significantly increased by 107 and 67% respectively, although no changes were measured in more caudal areas of cortex or in subcortical regions following MDMA. The limited autoradiographic study confirmed the cortical hyperaemia following MDMA, but the greater spatial resolution afforded by autoradiographic imaging suggested that the effect was most marked at boundary zones between the territories of anterior and middle cerebral arteries.

Acute exposure to MDMA produces a focal uncoupling of LCBF from metabolic demand in frontal and parietal cortices in conscious rat brain and the hyperaemia observed in this study is similar in anatomical distribution to the intracerebral haemorrhage reported in man, suggesting that the same mechanism (failure of cerebrovascular autoregulation) might underlie both phenomena.

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319P EVIDENCE TO DEMONSTRATE THAT POSTSYNAPTIC α_2 ADRENOCEPTORS IN THE RAT CORTEX ARE OF THE α_{2D} SUBTYPE

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Recently, Millan et al. (1993) have presented data to suggest that rat cortical α_2 adrenoceptors are of the α_{2D} subtype. However, central α_2 adrenoceptors are heterogeneous in terms of their synaptic location, ie they occur both pre- and postsynaptically. We have shown that the ratio of presynaptic : postsynaptic receptors in rat cortex is approximately 20 : 80 (Heal et al., 1993). This finding was revealed by quantifying postsynaptic α_2 adrenoceptors in rat cortex 3 days after destruction of noradrenergic neurones with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) and we have now employed this approach to define the subtype(s) of these receptors.

Male CD rats (180-200 g) were injected with DSP-4 (100 mg/kg, ip) 30 min after zimeldine (10 mg/kg, ip). Three days later, rats were killed and cortices removed. Inhibition constants (Ki values) were determined on pooled cortical membranes using [3H]RX 821002 (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline; 1 nM) defined by phentolamine (5 µM). Brain monoamines were measured in a random sample of six right cortices by h.p.l.c. with electrochemical detection.

Table 1 Inhibition constants of various compounds for postsynaptic α_2 adrenoceptors in rat cortex.

	Ki (nM)	Hill slope		Ki (nM)	Hill slope		Ki (nM)	Hill slope
Idazoxan	2.40	1.2	Rauwolscine	85.0	1.1	UK 14,304	274	0.9
Phentolamine	9.69	1.1	Yohimbine	105	1.0	(-)Epinephrine	364	0.8
Oxymetazoline	23.4	1.0	WB 4101	128	1.2	Prazosin	377	0.8
Clonidine	61.6	1.0	Spiroxatrine	182	1.1	Corynanthine	7533	1.0

Values are means of 3 - 4 determinations.

DSP-4 lesioning reduced cortical noradrenaline concentrations by 99 ± 1% ($P < 0.001$) with no significant effect on dopamine or 5-HT. Comparison of our Ki values (Table 1) with published data for α_{2A} (rabbit spleen), α_{2B} (rat kidney) and α_{2D} (rat submaxillary gland) adrenoceptors taken from Michel et al. (1989) and α_{2C} (OK cell line) adrenoceptors taken from Bylund et al. (1991) demonstrated a highly significant correlation with the α_{2D} subtype ($r = 0.920$; $P < 0.001$). Poor, non-significant correlations were obtained for the α_{2A} ($r = 0.479$), α_{2B} ($r = 0.305$) and α_{2C} ($r = 0.282$) subtypes. This point is emphasised by the low Ki values which we obtained for prazosin and rauwolscine (Table 1), as these ligands are reported to have high affinities for α_{2B}/α_{2C} and $\alpha_{2A}/\alpha_{2B}/\alpha_{2C}$ adrenoceptors, respectively (Michel et al., 1989; Bylund et al., 1991). In conclusion, therefore, the results presented argue strongly that postsynaptic α_2-adrenoceptors in rat cortex are predominantly, if not exclusively, of the α_{2D} subtype.

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320P AFFINITY AND SELECTIVITY OF BU224 AND BU239 FOR RABBIT BRAIN NON-ADRENOCEPTOR IDAZOXAN BINDING SITES (I_2-SITES)

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The α_2-adrenoceptor antagonist idazoxan (IDX) also binds to non-adrenoceptor idazoxan binding sites (NAIBS). These have recently been designated I_2-sites (Ernsberger., 1992). The function of these sites remains unclear, although they are downregulated in rat brain following chronic treatment with the MAO inhibitor clorgyline (Olmos *et al.*, 1993), and are possibly associated with an ATP-dependent K^+ channel (Ibbotson *et al.*, 1993). Lack of selective ligands has hindered the search for a definitive function of I_2-sites, although selectivity has been reported for RX821029 (2-(1,3-benzodioxanyl)-2-imidazoline) (Hudson *et al.*, 1992). We have now investigated the affinities of BU224 (2-(4,5-dihydroimidaz-2-yl)-quinoline-HCl) and BU239 (2-(4,5-dihydroimidaz-2-yl)-quinoxaline HCl) at α2-adrenoceptors and I_2-sites in rabbit whole brain membranes, using radioligand binding.

[^{3}H]-RX821002 (1-2 nM) and [^{3}H]-IDX (2-3 nM, in the presence of 5 µM rauwolscine) were used to label α_2-adrenoceptors and I_2 sites respectively. Membranes with [^{3}H]-ligand and displacing drug were incubated (50mM Tris-HCl buffer, pH 7.4) in triplicate to equilibrium (30min, final volume 1ml, 22°C). Bound and free radioactivity were separated by filtration. Specific binding of [^{3}H]-RX821002 and [^{3}H]-IDX was defined in the presence of 10µM rauwolscine and 10 µM 6-fluoro-idazoxan (RX801023) respectively. The results are shown in Table 1 below (mean ± s.e.mean; n = 4 - 6 experiments).

Table 1	Ki (nM)		
	I_2-sites	α2-receptors	α2/I_2-site
Idazoxan	10.6 ± 1.4	55.4 ± 4.9	5.2
RX821002	>30,000	6.9 ± 0.2	>0.0001
RX801023	4.9 ± 0.5	142.8 ± 8.7	29.1
RX821029	2.7 ± 0.49	436.2 ± 26.0	161.5
BU224	2.9 ± 0.29	12100 ± 640	4172
BU239	1.9 ± 0.31	7296 ± 203	3840

Both BU224 and BU239 displayed high affinity and selectivity for I_2-sites with BU239 demonstrating 5-fold higher affinity for I_2-sites than idazoxan. Whilst BU224 and BU239 have comparable affinity to RX801023 and RX821029 with respect to I_2 sites, they offer considerably improved selectivity with respect to α2-adrenoceptors. We propose these new ligands will prove useful to probe the pharmacology and function of I_2-sites in the mammalian CNS. Their selectivity with regards to other receptor systems is presently under investigation.

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321P EFFECT OF INCORPORATION OF PHOSPHATIDYLETHANOL ON RELEASE OF NORADRENALINE FROM SH-SY5Y HUMAN NEUROBLASTOMA CELLS

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Phosphatidylethanol (PEt), the transphosphatidylation product of phospholipase D in the presence of ethanol, is an abnormal phospholipid found in the organs, specifically the brain, of ethanol treated rats (Alling et al., 1984). Phospholipase D, an enzyme increasingly implicated in signal transduction, has been identified in a variety of neuronal preparations. The aim of this study was to determine whether incorporation of this lipid affected [^{3}H]noradrenaline (NA) release by SH-SY5Y neuroblastoma cells, and hence whether PEt might be a mediator of ethanol induced neuronal dysfunction.

Undifferentiated SH-SY5Y cells, which have been described as a model of neurotransmitter release (Murphy et al., 1991), were used between passage 75-78 on 24 well multi-plates. Cells were labelled for 1h with [^{3}H]NA (2μM/ml, 50nM) in balanced salt solution (BSS) with pargyline (0.2mM) and ascorbic acid (0.2mM) and then washed with 0.3ml of balanced salt solution (BSS) every 15 min for 45 min. Cells were exposed to PEt in this period by washing with BSS containing 0.1mg/ml PEt (133μM), suspended by sonication. The same suspension containing [3H]PEt was applied to cells not labelled with [3H]NA in order to quantify incorporation and monitor breakdown. Cells were stimulated with carbachol (1mM), elevated extracellular potassium (100mM, with compensatory reduction in sodium) and the calcium ionophore ionomycin (5μM). Stimulating solution (0.5ml) was removed for scintillation counting after 3 min and unreleased [^{3}H]NA was extracted with perchloric acid (0.4M). Release of [^{3}H]NA was expressed as a percentage of total(released + unreleased).

Basal release was found to be most sensitive to the presence of PEt, falling to 58.76 $\pm$ 7.36% of control (n=5). Carbachol stimulated release was reduced to 77.54 $\pm$ 7.90% (n=5) of control values while stimulation with 100mM K^+ was less sensitive and was reduced by PEt to 88.16 $\pm$ 4.36% (n=3) of controls. However, ionomycin stimulated release was unaffected, 103.82 $\pm$ 5.37% (n=3), of control. PEt incorporation was found to amount to 13.48 $\pm$ 4.93% (n=5) of lipid phosphorus. Accumulation of [^{3}H]phosphatidic acid, a proposed metabolite of PEt (Metz and Dunlop, 1991) was determined by thin layer chromatography and found not to occur even after 2h of [^{3}H]PEt exposure.

These data show that PEt has an inhibitory effect on both agonist and depolarisation stimulated release of [^{3}H]NA and that these two mechanisms are affected with differing sensitivities. The failure of PEt to affect ionomycin stimulated release suggested that this phospholipid does not interfere with vesicle fusion and so must exert its inhibitory actions at other points in signal transduction.

This work was supported by the Wellcome Trust.

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322P NEUROENDOCRINE RESPONSES TO ROLIPRAM, DENBUFYLLINE AND 1,3-DICYCLOPROPYLMETHYL-8-AMINO-XANTHINE (BRL 61063) IN THE RAT

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The alkylxanthine denbufylline, a type IV phosphodiesterase (PDE) inhibitor with weak adenosine A_1 receptor blocking activity, stimulates the secretion of corticotrophin (ACTH), corticosterone (B) and luteinising hormone (LH) when given orally (Buckingham, J.C., Flack, J.D., Hadley, A.J. & Osbourne, J. unpublished observations.). In order to elucidate its site of action and the relative contribution of each of its actions to the secretory responses, we have compared the effects of denbufylline, given centrally (3μl into the third ventricle of conscious rats via indwelling cannulae placed under pentobarbitone anaesthesia) and peripherally (i.p.), on the secretion of corticosterone and LH in adult male CFY rats with those of two further selective type IV PDE inhibitors, rolipram and BRL 61063, which have negligible and significant A_1 receptor blocking activity respectively. In addition we have investigated the effects of these drugs on the release of ACTH and LH from rat pituitary segments in vitro (Hadley et al., 1990). Hormones were measured by radioimmunoassay and the results expressed as ng/ml serum or for in vitro experiments, ng/mg tissue; values represent mean $\pm$ SEM (n=4-6) with control (vehicle) values shown in parenthesis.

Given peripherally, rolipram (8-200μg/kg i.p.), denbufylline (70-600μg/kg i.p.) and BRL 61063 (0.25-30 μg/kg i.p.) produced significant, dose-dependent increases in pituitary adrenocortical activity; thus at the highest doses tested the three drugs raised the serum corticosterone concentrations to 90 $\pm$ 6 (vs. 11.2 $\pm$ 3.5) $p<0.005$, 80 $\pm$ 10 (vs. 4.4 $\pm$ 0.5) $p<0.005$ and 127 $\pm$ 45 (vs. 3.3 $\pm$ 1.4) $p<0.005$, respectively. No such effects were evident when rolipram (8ng/kg-1μg/kg) or denbufylline (0.05-1μg/kg) were given i.c.v. although BRL 61063 (1-600 ng/kg i.c.v.) produced a modest increase in steroid secretion [at 600 ng/kg B = 22 $\pm$ 4 (vs. 5 $\pm$ 2.1) $p<0.01$]. The serum LH concentration was unaffected by rolipram given centrally (8ng-1μg/kg, i.c.v) or peripherally (8-200 μg/kg i.p.), by denbufylline (0.05-1 μg/kg i.c.v.) or by BRL 61063 (0.25-30 μg/kg i.p.). However, when given i.p., denbufylline (70-600 μg/kg) produced a small increase in serum LH [at 600μg/kg: LH = 1.2 $\pm$ 0.3 (vs. 0.6 $\pm$ 0.1) $p<0.05$] as also did BRL 61063 when injected i.c.v. although only at a dose of 120 ng/kg [0.42 $\pm$ 0.07 (vs. 0.26 $\pm$ 0.04) $p<0.05$].

In vitro, rolipram (100μM), denbufylline (0.01-1mM) and BRL 61063 (10-100μM) increased the release of ACTH from rat anterior pituitary segments [100μM rolipram: ACTH = 0.66 $\pm$ 0.08 (vs. 0.39 $\pm$ 0.04), $p<0.05$, 1mM denbufylline ACTH = 10.27 $\pm$ 2.33 (vs. 5.39 $\pm$ 0.67) and BRL 61063 (100μM) ACTH = 1.1 $\pm$ 0.31 (vs. 0.35 $\pm$ 0.16) $p<0.05$]. Denbufylline caused small but significant increases in the spontaneous LH release [LH: 134 $\pm$ 9.9 (vs. 68 $\pm$ 7.5) $p<0.05$] but rolipram (0.1-1 μM) and BRL 61063 (0.01-100μM) did not.

The results support our previous suggestion (Hadley et al., 1990) that the ACTH and LH responses to denbufylline are effected primarily at the pituitary level by mechanisms involving PDE IV inhibition. The finding that BRL 61063, unlike rolipram or denbufylline, stimulated the release of corticosterone when given i.c.v. raises the possibility that adenosine receptors may contribute to the control of neuroendocrine function at the hypothalamic level.

We are grateful to SmithKline Beecham Pharmaceuticals for financial support.

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323P THE EFFECTS OF 2-CHLORO-N^6-CYCLOPENTYLADENOSINE (CCPA) ON SPONTANEOUS MINIATURE EXCITATORY POSTSYNAPTIC POTENTIALS IN RAT HIPPOCAMPAL CA1 NEURONES

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It has been suggested (Lupica *et al*, 1992) that adenosine inhibits synaptic transmission by reducing the probability of release from the presynaptic terminals, rather than by a postsynaptic action. Adenosine A1 receptors are present on the terminals of Schaeffer collateral commisural neurones and on CA1 pyramidal neurones (Dunwiddie & Fredholm, 1989).

Blind whole cell patch current clamp recordings were made from hippocampal CA1 neurones contained in a 450μm thick hippocampal slice prepared from 50-100g rats. The patch electrode contained (mM): K-Gluconate 130, KCl 10, NaCl 5, EGTA 10, HEPES 10, $CaCl_2$ 1, $MgCl_2$ 2, $NaHCO_3$ 2, Na_2ATP 2, pH 7.4 with KOH and the fluid bathing the slices (flow rate 2-3ml/min) contained (mM): NaCl 126, $NaHCO_3$ 26, D-Glucose 10, KCl 2.75, $NaH_2PO_4.2H_2O$ 1.25, $MgSO_4.7H_2O$ 2, $CaCl_2$ 2.5, picrotoxin 0.1, gassed with 95%O_2/5%CO_2 at room temperature. EPSPs were evoked by stimulating electrodes placed on the Stratum Radiatum 1-2mm from the CA1 region. Hippocampal CA1 neurones were identified by a calcium spike evoked by a large depolarizing current pulse and an inwardly rectifying sag produced by a large hyperpolarizing current pulse. In the spontaneous release experiments the ACSF contained tetrodotoxin (TTX, 100nM). The amplitude and interval distributions and frequency of the spontaneous miniature events were measured using Spike 2 (CED, Cambridge).

2-Chloro-N^6-cyclopentyladenosine (CCPA 0.1 - 10μM, n = 5) inhibited the evoked EPSP by 35-50% but did not change the input resistance of the CA1 neurone. This is suggestive of a pre- rather than a postsynaptic action. Studies of spontaneous miniature EPSPs confirmed this locus of action. CCPA (100nM, n = 7) reduced the frequency of the spontaneous events by 43% from 0.984 ± 0.238Hz to 0.558 ± 0.091Hz (mean ± S.E.M., $P < 0.05$). The mean of the interval distribution was increased by 54% from 1.38 ± 0.304s to 2.13 ± 0.393s (mean ± S.E.M., $P < 0.01$). The mean of the amplitude distribution was unaltered.

8-Cyclopentyl-1,3-dipropylxanthine (DPCPX 30nM, n = 3), an A1 selective antagonist, completely reversed the effects of CCPA. Over the time course of these experiments there appeared to be little run down in the frequency of the miniature EPSPs as DPCPX returned them to control levels. In the absence of CCPA, DPCPX increased the frequency of the spontaneous miniature EPSPs by 62% from 0.621 ± 0.069Hz to 1.005 ± 0.119Hz. This finding raises the possibility that the synaptic activity in the slice is modulated by endogenous adenosine released into the extracellular space in the acute hippocampal slice. In cultured hippocampal slices Thompson *et al*, 1992, found DPCPX to be without effect. Thus it is possible that adenosine only enters the extracellular space following mechanical damage to the tissue.

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324P MODULATION OF POSTSYNAPTIC ADENOSINE SENSITIVITY IN CALCIUM-FREE MEDIA IN RAT HIPPOCAMPAL SLICES

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We previously showed that adenosine postsynaptic sensitivity was lost in calcium-free medium and that raising the concentration of divalent cations restored adenosine responses (Hosseinzadeh and Stone, 1993). We have now considered the mechanism of this effect.

Hippocampal slices 450 μm thick were prepared from male Wistar rats (170-210 g) and superfused at 30°C with ACSF (in mM: KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 10) saturated with 95% O_2 / 5% CO_2. Orthodromic extracellular recordings were made in the CA1 pyramidal cell layer following stimulation of Schaffer collateral fibres in stratum radiatum. Stimulation of the alveus was used for antidromic stimulation. In antidromic stimulation experiments, $CaCl_2$ was omitted or reduced to 0.1 mM.

Adenosine analogues, R-PIA and 2-chloroadenosine, abolished the orthodromic potential at 50 nM and 1 μM respectively, but even high concentrations i.e. 50 μM R-PIA and 500 μM 2-chloroadenosine had no effect on the antidromic bursts in calcium-free medium. Kynurenic acid, a non-specific excitatory amino acid antagonist, abolished the population spike at 1 mM in normal ACSF, but even 5 mM had no effect on the antidromic potentials in the absence of calcium. Baclofen at 20 μM decreased secondary spike size by 48.48% ±5.65 (mean ± s.e.m., $p<0.001$, n=21). Even in the presence of baclofen adenosine up to 200 μM (n.s., $p=0.086$, n=8) did not decrease the antidromic potential size more than baclofen alone. Because omitting calcium destabilizes the cell membrane, carbamazepine was added to reduce neuronal excitability. Carbamazepine 50 and 100 μM decreased secondary spike size respectively by 6.67% ± 3.74 (n.s, $p=0.108$, n=10) and 21.47% ±4.91 ($p=0.05$, n=4) respectively. Although 100 μM adenosine had no effect alone on the secondary spike size, combination with carbamazepine at 50 and 100 μM decrease spike size by 55.52% ± 9.63 ($p<0.01$, n=6) and 74.08% ± 12.31 ($p<0.01$, n=5) respectively. Carbamazepine at a concentration of 50 μM did not increase the inhibitory effect of 20 μM baclofen ($p=0.214$, n=4). Since carbamazepine may be an adenosine antagonist (Skerritt et al., 1983; Stone, 1988), theophylline was also studied. Theophylline 10-50 μM did not restore the inhibitory effect of adenosine 200 μM and surprisingly theophylline 50 μM alone decreased the secondary spike size by 50.61% ± 6.79 ($p<0.001$, n=14). Adding 0.1 mM calcium to the calcium-free medium had no effect on secondary spike size alone but significantly decreased the inhibitory effect of theophylline by 16.3% ± 1.49 ($p\leq0.001$, n=5). Thapsigargin 1 μM, an inhibitor of calcium-activated ATPase, which elevates intracellular calcium level, did not restore adenosine sensitivity in calcium-free medium.

The results show that loss of adenosine sensitivity in calcium-free medium is not likely to be due to a change of uptake or metabolism of adenosine or release of excitatory amino acids. Further, It seems that the postsynaptic actions of adenosine differ from those of baclofen and are dependent on the stability of the cell membrane.

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325P FUNCTIONAL CHARACTERISATION OF A HUMAN HIPPOCAMPAL ADENOSINE A_1 RECEPTOR

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A human hippocampal adenosine receptor has been recently cloned, transfected and expressed in a Chinese hamster ovary (CHO) cell line (Townsend-Nicholson and Shine, 1992). These authors reported that the adenosine A_1 receptor-selective agonist cyclopentyladenosine (CPA) caused dose-dependent reductions in forskolin-stimulated intracellular cyclic AMP concentrations, whilst radioligand binding studies showed that the adenosine A_1 receptor-selective antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), exhibited high affinity for the receptor. In the present study, we have investigated the effects of a range of adenosine receptor ligands in order to characterize further the pharmacological properties of this receptor.

Experiments were carried out on transfected CHO cells initially plated out at a density of $1x10^5$ cells/well in medium (1:1 mixture of Dulbecco's modified Eagle medium (DMEM) and HAMS F-12) containing adenosine deaminase (2u/ml) and the phosphodiesterase inhibitor rolipram (0.1mM). Adenylate cyclase activity was stimulated for 10min with a combination of prostaglandin E_2 (10μM) and forskolin (0.1μM). Adenosine analogues were added 10min prior to cyclic AMP stimulation. Pertussis toxin was preincubated for 3h respectively before analogues were added. The response was terminated by the addition of 0.2M HCl and cyclic AMP assayed in the resulting supernatant using a radioimmunoassay kit (Rianen Assay System).

The non-selective adenosine receptor agonist 5'-N-ethylcarboxamidoadenosine (NECA) and the adenosine A_1 receptor selective agonists CPA and N-(1S-trans)-[2-hydroxycyclopentyl]-adenosine, (GR79236; Gurden et al.,1993) produced concentration-dependent inhibition of intracellular cyclic AMP accumulation. IC_{50} values for 50% inhibition of maximum stimulated cyclic AMP concentrations were $8x10^{-10}$M, $1x10^{-9}$M and $6x10^{-9}$M for CPA, NECA and GR79236 respectively. GR79236-mediated inhibition of cyclic AMP accumulation was antagonised by DPCPX in a concentration-dependent manner (CR=16.6 at $3x10^{-8}$M). Pertussis toxin (25-75ng/ml) caused concentration-dependent blockade of the inhibitory effects of GR79236, abolishing the response at the highest concentration. The adenosine A_{2a} receptor-selective agonist CGS-21680 and the adenosine A_{2b} receptor agonist, metrifudil, only inhibited cyclic AMP accumulation at high concentrations (IC_{50} values of $2x10^{-7}$M and $6x10^{-6}$M respectively).

Our results confirm the presence of an adenosine A_1 receptor on this cell line. The potency of DPCPX and the rank order of agonist activity are consistent with an A_1 receptor. The receptor mediates an inhibition of intracellular cyclic AMP generation via a pertussis toxin-sensitive inhibitory G-protein (G_i).

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326P REGIONAL AUTORADIOGRAPHIC LOCALISATION OF HIGH AFFINITY [^{3}H]α,β-METHYLENE ATP BINDING SITES IN THE RAT BRAIN

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Purinoceptors can be divided into subtypes based on the differential affinities of a range of agonists (Burnstock, 1991). One of these agonists, α,β-methylene ATP (α,β-meATP), has high affinity and selectivity for the P_{2x} purinoceptor and is therefore useful to identify this receptor subtype (Burnstock, 1991). We have previously shown that [^{3}H]α,β-meATP binding sites are present in a wide range of central and peripheral tissues and that the pharmacology of these binding sites is in accordance with the ligand binding to a P_{2x} purinoceptor (Michel & Humphrey, 1993). This study was carried out to determine the detailed distribution of [^{3}H]α,β-meATP binding sites in the rat central nervous system (CNS) and to identify regions containing high levels of specific binding for future functional studies.

Frozen sections (20 μm) of unfixed brain and spinal cord (n = 3 rats), on coated slides, were preincubated in 50 mM Tris.HCl and 3 mM $CaCl_2$ buffer, pH 7.4 for 40 min at 4°C, then incubated for 40 min at 4°C with ~1.5 nM [^{3}H]α,β-meATP (Michel & Humphrey, 1993) with or without 0.3 μM α,β-meATP for definition of high affinity binding, washed for 3 x 10 min in ice-cold buffer and dipped for 10 sec in ice-cold distilled water. The dried slides were apposed to X-ray film for 2 months and the images were quantified using appropriate standards.

The binding of [^{3}H]α,β-meATP to the rat CNS appeared generally diffuse. However, it was possible to quantify localised binding in a number of regions. The highest levels of specific binding (grey values, mean ± s.e. mean, n ≥ 10 per rat) were found in the lumbar (0.085 ± 0.015) and thoracic spinal cord (0.081 ± 0.011) and significant binding was seen in the following brain areas: facial nucleus (0.083 ± 0.011), olfactory bulb (0.079 ± 0.008), dorsal raphe nucleus (0.076 ± 0.019), granule cell layer of the cerebellum (0.067 ± 0.018), superior grey layer of the superior colliculus (0.051 ± 0.013), substantia nigra (0.049 ± 0.006), subiculum (0.043 ± 0.009), dentate gyrus (0.041 ± 0.014), cortex (0.041 ± 0.004), striatum (0.031 ± 0.013). There appeared to be few or no specific binding sites in the pons and medulla.

[^{3}H]α,β-meATP binds to a number of regions in the rat CNS, in particular to the thoracic and lumbar spinal cord. However, the binding was relatively homogeneous, e.g. in the grey matter of the spinal cord where no particular region contained higher levels of binding. This could be due to these binding sites being on glial cells rather than neurones. In addition, "non-specific" binding was high, probably a reflection of the high density of the low affinity sites for this ligand (Michel and Humphrey, 1993). Electrophysiological studies have suggested the presence of P_{2x} purinoceptors in the medial habenula (Edwards et al., 1992) but none were seen in this nucleus in this study. Overall, our findings suggest that the spinal cord might be worthy of study for the presence of functional P_{2x} purinoceptors.

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327P ROLE OF cAMP IN A_{2A} RECEPTOR MEDIATED STIMULATION OF [^{3}H]-ACh RELEASE FROM RAT STRIATAL SYNAPTOSOMES

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We have previously shown that the evoked release of [^{3}H]-ACh from rat striatal synaptosomes is stimulated by the selective A_{2a} adenosine receptor agonist, CGS 21680 (Kirkpatrick & Richardson, 1993). Characteristically this receptor is positively coupled to adenylate cyclase (Van Calker et al., 1979) and in the striatum, stimulation of this receptor has been shown to increase cAMP production (Brown *et al.*, 1990). However, we have recently shown that stimulation of the A_{2a} receptor decreased the release of [^{3}H]-GABA from striatal synaptosomes and that this inhibition was reversed by rises in the intracellular concentration of cAMP (Kirk & Richardson, 1993). Therefore, in this study we were keen to investigate the role of cAMP in the A_{2a} mediated enhancement of [^{3}H]-ACh release from rat striatal synaptosomes.

After loading with 2.5 μCi ml^{-1} [methyl-^{3}H]choline chloride the synaptosomes were drawn onto a series of eight parallel perfusion chambers containing Whatman GF/B filters and superfused with buffer at 0.5 ml min^{-1}, maintained at 37°C and aerated with 95 % O_2 + 5 % CO_2. After an equilibration period of 40 min superfusate samples were collected at 2 min intervals into scintillation vials containing 10 ml Emulsifier safe scintillant for scintillation counting. Release of [^{3}H]-ACh was evoked during two 90 s periods of depolarisation (S_1 and S_2) with 15 mM KCl and the release expressed as a percentage of the total radioactivity present at the point of release. S_1 included elevated KCl (15 mM) only whereas S_2 contained either 15 mM KCl alone (control) or with the drugs under investigation. The results are expressed as percentage modulation of [^{3}H]-ACh release when compared to control and are expressed as mean ± sem.

Perfusion of synaptosomes with CGS 21680 (0.1 nM) significantly enhanced the evoked release of [^{3}H]-ACh by 52.8 ± 10.4 % (n=6). This stimulation was mimicked by the adenylate cyclase stimulant forskolin (2 μM) which enhanced the evoked efflux by 52.9 ± 12.7 % (n=4) and by the cAMP analogue, 8 bromo cAMP (1 mM) which caused an increase of 33.2 ± 9.2 % (n=5). It is unlikely that the effects of forskolin were non specific since the inactive derivative of forskolin, 1,9 dideoxyforskolin (2 μM), did not enhance the release of [^{3}H]-ACh. Perfusion with the protein kinase A antagonist, HA 1004 (10 μM) for 6 min prior to and during depolarisation had no significant effect on the evoked release of ACh. However, HA 1004 significantly reduced the ability of forskolin to enhance the release of [^{3}H]-ACh; release in the presence of both HA 1004 and forskolin was not significantly different from that seen in the presence of HA 1004 alone (n=3). In contrast, HA 1004 (10 μM) did not significantly impair the ability of the A_{2a} agonist CGS 21680 (0.1 nM) to enhance the release of [^{3}H]-ACh, the evoked efflux was increased by 52.2 ± 8.68 % (n=6).

These results suggest, that increasing intracellular concentrations of cAMP can, via a protein kinase A dependent mechanism, enhance the release of [^{3}H]-ACh from rat striatal synaptosomes. However, the A_{2a} receptor appears to increase the efflux of [^{3}H]-ACh by an alternative method.

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328P DIFFERENTIAL *IN VIVO* ACETYLCHOLINE RELEASE BETWEEN HEMISPHERES IN THE RAT HIPPOCAMPUS

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Recent clinical studies have demonstrated short term memory changes after unilateral temporal lobectomy or unilateral amygdalo-hippocampectomy for the relief of tempory lobe epilepsy. Left side surgery produced clear deterioration in some cognitive tests whilst right side surgery produced some improvement (Goldstein & Polkey, 1993). It may be pertinent to laterality in animal models of cognition that studies in the rat have reported the existence of differential *ex vivo* levels of 5-HT in the right and left hemispheres of the frontal cortex and hippocampus (Cheng *et al.*, 1993). The present study, using the *in vivo* microdialysis technique, investigates whether similar interhemispheric differences exist in the release of acetylcholine (Ach) in the hippocampus of the rat.

Microdialysis probes (4 mm dialysis membrane) were implanted into chronically indwelling bilateral cannula located in the hippocampus (co-ordinates: Ant +2.0, Vert +8.0, Lat 4.8; Paxinos & Watson, 1982) of male Hooded Lister rats (375-500 g) 16 hours prior to the experiment. Probes were perfused with artificial cerebrospinal fluid containing 2.0 μM neostigmine at a rate of 2.0 μl min^{-1}. After a 100 min stabilisation period, 20 min samples were collected and immediately analysed for Ach by HPLC-ECD in conjunction with pre-choline oxidase and post-choline oxidase/Ach esterase enzyme reactors.

The basal Ach levels ranged from 31.7 to 307.8 pg 40μl^{-1} in the right hippocampus and 10.0 to 318.2 pg 40μl^{-1} in the left (Figure1). Among the 22 animals investigated, 4 had less than a 20 % difference in the Ach levels between the right and left hippocampus, 8 had 33-240% higher levels in left hemisphere than the right whilst 10 had 32-1790% greater levels in the right hippocampus.

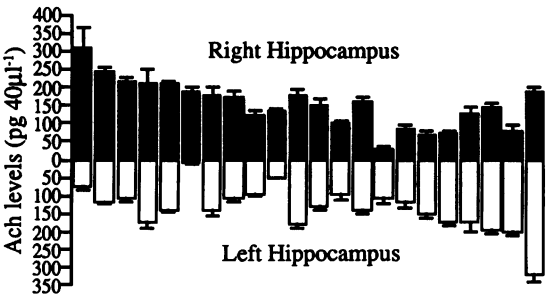


Figure 1 Extracellular levels of Ach in the right and left hippocampus of 22 rats. Each value represents the mean (±S.E.M.) of the basal levels obtained from five determinations in each rat.

The results from the present study indicate a lateralised basal release of Ach in the hippocampus of the majority of animals in either the right or left hemisphere. The findings are significant to the experimental design of sampling of Ach in microdialysis experiments and question whether lateralised Ach release may exist in other brain structures. The significance of the findings to cognitive performance and the effect of drugs on cholinergic function remains to be determined.

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329P THE EFFECT OF SCOPOLAMINE ON THE ACQUISITION OF COCAINE-INDUCED CONDITIONED PLACE PREFERENCE IN THE MOUSE

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The conditioned place preference (CPP) paradigm is based on the assumption that environmental neutral cues can take on secondary reinforcing properties when paired with a reinforcing drug (Spyraki, 1988). Drugs, co-administered at the time of conditioning, which attenuate or antagonise this response, are presumed to have antagonised the reinforcing properties of that drug. However, it is not known to what degree the acquisition of the mouse CPP response could be influenced by interference with cognitive processes. The present study therefore examined the influence of scopolamine, a centrally acting cholinergic antagonist, which has been widely used to produce cognitive deficits in a number of animal models (Salamone et al, 1987) on the acquisition of cocaine-induced CPP in the mouse.

Briefly four automated three chambered boxes and male adult BKW mice were used in the experiment. The protocol and apparatus has previously been described (Marshall et al, 1992) except a pretreatment of scopolamine (0.5 mg/kg i.p.) or saline was used, 30 min prior to cocaine (5mg/kg s.c.) or 0.9% saline (1ml/100g) conditioning. The time spent in each chamber , pre- and post-conditioning, was used as a measure of preference responding. Data were analysed by two factorial analysis of variance.

Saline/saline paired animals showed no significant effects of conditioning, and this was irrespective of the particular pretreatment, either saline ($F(2,16) = 0.636$, $p=0.5423$) or scopolamine ($F(2,18) = 1.101$, $p=0.3539$). The group pretreated with saline and conditioned with cocaine, showed a significant change in preference behaviour ($F(2,18) = 7.014$, $p = 0.0056$). This was associated with a significant increase in time spent on the drug paired side from 312.4±17.8 s to 401.7±27.9 s ($p<0.05$, post hoc t test). Mice which were pretreated with scopolamine prior to cocaine conditioning, did exhibit a change in preference behaviour in the subsequent post-conditioning test ($F(2, 18)= 3.758$, $p = 0.0433$). However, even though there was an increase in time spent on the cocaine conditioned side, from 305.8±12.9 s to 345.1±17.8 s, this failed to reach significance ($p>0.05$).

These results indicate that scopolamine can attenuate the acquisition of the CPP response to cocaine in the mouse at a dose which has been previously shown to influence cognitive processes (Salamone et al, 1987), demonstrating the importance of such processes in the acquisition of the CPP response. Additionally, these data indicate that care should be taken in the interpretation of studies which examine the antagonism of a CPP response.

CT was supported by a Wellcome Vacation Studentship; JWBM is supported by Glaxo Group Research.

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330P THE EFFECTS OF CAPTOPRIL AND ITS WITHDRAWAL ON RAT BEHAVIOUR IN 8-ARM RADIAL MAZE TESTS OF LEARNING AND MEMORY

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Studies in both humans (Currie et al 1990) and animals (Barnes et al 1989) suggest that the angiotensin converting enzyme (ACE) inhibitor, captopril (CAP), may enhance performance in a number of tests of cognitive function. This study examined the possibility that the administration of captopril during acquisition of a test of learning and memory might render the animals dependent upon the drug for the successful performance of the task.

In the first experiment, water-deprived (20h) rats were trained (1 training trial/day on the 5 working days of each week) to locate the 4 arms baited with sweetened water in an 8-arm radial maze. Thirty minutes prior to each training session the animals (n=8 per group) were given IP injections of saline (SAL) or CAP (0.25 or 1mg/kg). When the animals had reached asymptotic levels of performance (24 training days), the effects of scopolamine (SCOP; 0.033 or 0.1mg/kg IP) and/or CAP-withdrawal (SAL in place of CAP on the test day) were examined for their effects on performance of the task. In the second experiment all 8 arms of the maze were baited. Four groups of rats (SAL/SAL; CAP/SAL; SAL/SCOP; CAP/SCOP; n=6-8 per group) were given IP injections of SAL or CAP (1mg/kg) 30 minutes prior to each training session followed by SAL or SCOP (0.033mg/kg IP) 10 minutes prior to each training session. After 24 training days, the effects of withdrawing CAP and/or SCOP were examined by injecting saline in place of the drug solutions on the test day. In the first experiment neither dose of CAP tested influenced acquisition of the task although, at asymptotic levels of performance CAP (1mg/kg) decreased ($P<0.05$) re-entries into baited arms from 1.00 ± 0.57 to 0.1 ± 0.01. CAP-withdrawal had no effects on maze behaviour. In the SAL-trained rats SCOP (0.1mg/kg) increased ($P<0.05$) entries into the unbaited arms from 1.13 ± 0.40 to 2.13 ± 0.52 but had no significant effects on re-entries into the baited arms. Neither CAP nor its withdrawal influenced the response to SCOP. In the second experiment, SCOP attenuated acquisition of task by both decreasing ($F(1,25)=35.5$; $P<0.001$) the number of entries made before one of the arms was re-entered and increasing ($F(1,15)=50.3$; $P<0.001$) the number of arms re-entered during the trial. At asymptotic levels of performance CAP-trained rats made fewer re-entries ($P<0.05$) into baited arms. In the CAP-withdrawn rats, re-entries were not different from control. At asymptotic levels of performance, SCOP increased re-entries from 4.63 ± 1.15 to 16.63 ± 3.06 ($P<0.001$) and decreased entries to repeat from 5.50 ± 0.71 to 4.25 ± 0.312 ($P<0.05$). These effects were not significantly attenuated by withdrawal of SCOP although there was a tendency for the number of re-entries to be reduced. Neither CAP administration nor its withdrawal evoked a significant effect on the responses evoked by SCOP or its withdrawal. The results suggest that CAP improves asymptotic levels of working memory (decreased re-entries into baited arms) but does not attenuate the impairments of performance evoked by acute or chronic treatment with SCOP. In CAP-withdrawn rats, maze performance returns to control levels and does not show enhanced sensitivity to the impairments evoked by SCOP.

JEG was supported by a studentship from the DDS Trust.

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331P INTRACEREBROVENTRICULAR (ICV) ADMINISTRATION OF THE CCK_A RECEPTOR ANTAGONIST A70104 INCREASES OPERANT FOOD INTAKE IN PIGS

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A70104 is a novel CCK_A receptor antagonist which is unable to enter the CNS from the systemic circulation (see Ebenezer and Parrott, 1993). We have recently shown that i.v. administration of A70104 has no effect on food intake in pigs (Ebenezer and Parrott, 1993). This constrasts with another CCK_A receptor antagonist, devazepide, which does increase food consumption in pigs after i.v. administration (Ebenezer et al., 1990). However, Baldwin and de la Riva (1991) have shown that devazepide, which crosses the blood brain barrier, can increase feeding by a central mode of action. Hence, the present study was undertaken to investigate whether intracerebroventricular (icv) administration of A70104 (provided by Dr. C.W. Lin, Abbott Laboratories, U.S.A.) would increase food intake in pigs.

Prepubertal large white pigs (b.w. 25 - 35 kg) were implanted under halothane anaesthesia with cannulae aimed at the lateral cerebral ventricle for icv injections. The pigs lived in metal cages and performed operant responses to obtain food and water. The pigs were allowed to make operant reponses for food between 10.00h - 10.30h in the morning and 14.30h - 16.00h in the afternoon each day. A buzzer signalled when the feeders were activated. The number of food reinforcements obtained by each pig was monitored on a chart recorder. Water was freely available at all times.

Experiment 1. The pigs (n=6) were injected icv with either vehicle solution or A70104 (50 and 100 μg) 10 min before the start of the afternoon feeding session. The number of food reinforcements obtained by each pig was measured for 60 min after the feeders were activated. A repeated measures design was used with each pig receiving all doses of A70104 or vehicle in a random fashion.

Experiment 2. The pigs (n=4) were injected icv with either vehicle solution (control), vehicle followed by CCK (1 μg), or A70104 (50 μg) followed by CCK (1 μg). The two injections were separated by 10 min, and the 2nd injection was given 10 min before the start of the afternoon feeding session. The number of food reinforcements obtained by each pig was measured for 60 min after the feeders were activated.

Analysis of the data obtained in Experiment 1 showed a significant main effect of treatment ($F_{2,10}$=14.149, P<0.01). Post-hoc tests revealed that A70104 (50 μg, icv) increased food intake from a mean control value of 308 $\pm$ 88g to 522 $\pm$ 68g (P<0.01). In contrast, the 100 μg dose had no effect on operant feeding (mean food intake $\pm$ s.e.m.: 342 $\pm$ 71g). Interestingly, in a pilot study, a lower dose of A70104 (i.e. 20 μg, icv) increased food intake in pigs by over 20% (unpublished results). In the 2nd experiment, CCK (1 μg) produced a significant depression in food intake from a control value of 278 $\pm$ 75g to 35 $\pm$ 21g (P<0.05). Pretreatment with A70104 (50 μg, icv) abolished the effects of CCK (1 μg, icv) on operant feeding. (Food intake after A70104 + CCK = 230 $\pm$ 31g). The results of this study extend previous observations (Baldwin and de la Riva, 1991), and suggest that a central CCK_A receptor mediated mechanism may play a physiological role in the regulation of feeding.

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332P PROFILE OF GR159897, A NON-PEPTIDE TACHYKININ NK_2 RECEPTOR ANTAGONIST, IN A PRIMATE INTRUDER RESPONSE MODEL

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Following infusion into the dorsal raphe nucleus of rats, peptidic tachykinin NK_2 receptor antagonists have been shown to release suppressed behaviours in a novel aversive environment, which is indicative of anxiolytic activity (Stratton et al., 1993). In the present study, we have examined the effects of GR159897 ((R)-1-[2-(5-fluoro-1H-indol-3-yl)ethyl]-4-methoxy-4-[(phenylsulfinyl)methyl]-piperidine), a novel, potent, non-peptide tachykinin NK_2 receptor antagonist (Ball et al., 1994), in a putative primate model of anxiety, the marmoset intruder response model (Costall et al., 1988). We have compared its effects to the structurally dissimilar non-peptide NK_2 receptor antagonist SR48968 (Emonds-Alt et al., 1992) and to chlordiazepoxide (CDP). The marmoset intruder response model is based on findings that, in the presence of a stranger ('threat'), positioned in front of the cage, marmosets will retreat to the back of the cage and posture towards the perceived threat. Anxiolytic agents, for example diazepam, increase the time spent at the front of the cage and decrease the number of postures displayed (Costall et al., 1988).

Common marmosets (Callithrix jacchus), housed as single sex pairs, were selected for each study on the basis of spending $\leq$ 25% (30sec) of a 2min period at the front of the cage on two occasions prior to study. Marmosets were injected subcutaneously with drug or vehicle (saline). Forty min later, time spent at the front of the cage and locomotor activity (LMA) were recorded by the observer for 2min. Locomotor activity was assessed by the number of jumps made by the animals between perches and between the back and front of the cage. Any overt behaviours were also noted. Data were analysed using a paired t-test. GR159897 (0.2-50μg.kg^{-1}s.c.) caused a significant (P<0.05) increase in the mean time ($\pm$s.e.mean) spent by marmosets at the front of the cage (e.g.10μg.kg^{-1}, 29$\pm$7s; vehicle 6$\pm$1s, n=6). Similar profiles of action were produced by ($\pm$)SR48968 (10-50μg.kg^{-1}s.c.) and CDP (0.3-3.0mg.kg^{-1}s.c.) (($\pm$)SR48968 at 10μg.kg^{-1}, 41$\pm$10s; vehicle 7$\pm$2s, n=7; CDP at 1mg.kg^{-1}, 61$\pm$12s, vehicle 9$\pm$2s, n=9). LMA did not differ from that of control animals. There were no other overt behaviours produced by GR159897 or ($\pm$)SR48968. Slight ataxia was observed in some of the animals which had received the highest dose (3mg.kg^{-1}) of CDP.

These results provide further evidence for an ability of NK_2 receptor antagonists to restore behaviours which have been suppressed by aversive conditions. Such effects imply a role for endogenous tachykinins in the mediation of anxiety-related behaviours.

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333P MEASUREMENT OF THE CNS PENETRATION OF TACHYKININ NK_1 RECEPTOR ANTAGONISTS IN THE GERBIL USING *EX VIVO* BINDING AND A SCRATCHING RESPONSE

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We have investigated the CNS penetration of the non-peptide NK_1 receptor antagonists CP99,994 and its less active enantiomer CP100,263 (Desai et al., 1992) in a [^{3}H]-substance P (SP) *ex vivo* binding assay in the gerbil forebrain and spinal cord. We have also compared these results with the ability of the antagonists to inhibit the caudally-directed biting and scratching response (CDBS) following intrathecal administration of the NK_1 agonist GR73632 (δAva[L-Pro^9,N-MeLeu^{10}]SP(7-11)).

Drugs were administered (s.c. in saline vehicle) to male Mongolian gerbils (40-70g). For the *ex vivo* binding assay, the gerbils were anaesthetised (5-120min after drug treatment) with a lethal dose of pentobarbitone and transcardially perfused with saline. The forebrain and, in some experiments, the spinal cord were removed and homogenised in Tris buffer (50mM,pH7.4) at a concentration of 60mg wet weight ml^{-1}. Specific [^{3}H]-SP (0.6nM) binding, defined with CP99,994 (1μM), was measured as previously described (Beresford et al., 1991). Results are expressed as a percentage of inhibition of binding compared to vehicle-treated controls (mean±s.e.mean; n≥3). ED_{50} values were determined from the dose-response curves using ALLFIT. CDBS was induced by direct (i.t.) administration of GR73632 (0.5-128pmol;5μl). Animals (12 per dose) were observed for 2min and scored for the presence or absence of CDBS. ED_{50} values were calculated from the dose-response curves in controls and in animals administered with the antagonists 15min prior to GR73632. Dose-ratios (with 95% confidence limits) were then determined.

CP99,994 (0.001-10mgkg^{-1}) potently and dose-dependently inhibited the binding of [^{3}H]-SP to forebrain membranes with an ED_{50} of 0.02mgkg^{-1} Similar effects were observed when the binding was measured in the spinal cord (ED_{50}=0.05mgkg^{-1}). Time course studies with CP99,994 (0.1mgkg^{-1}) indicated that the maximum effect was obtained 30 min after drug administration, after which time there was a progressive fall off in the effect (at 5,30,60,120min binding was inhibited by 42±13, 82±1, 76±2, 25±8% respectively). CP100,263 (1-30mgkg^{-1}) was 250-fold weaker at inhibiting binding to forebrain membranes (ED_{50}=5mgkg^{-1}). In the CDBS model, CP99,994 and CP100,263 (4mgkg^{-1}) caused dose-ratio shifts of 41.2 (15.6-122.4) and 2.1 (0.6-5.6) respectively. The high potency of CP99,994 in both the *ex vivo* binding and CDBS models is consistent with the high affinity of this compound at NK_1 receptors (gerbil cortex pKi=10.0) and indicates that it readily penetrates the CNS. In agreemeent with its lower *in vitro* potency at NK_1 receptors, CP100,263 (pKi=7.2) was weak in both the *ex vivo* binding and CDBS assays.

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334P AGONIST-STIMULATED Ins (1,4,5)P_3 ACCUMULATION AND Ca^{2+} MOBILIZATION FROM INTACT AND PERMEABILIZED CHO CELLS EXPRESSING RECOMBINANT MUSCARINIC RECEPTOR SUBTYPES

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It is now well established that the structurally related m1 and m3 muscarinic acetylcholine receptors are coupled to the activation of phospholipase C and the mobilization of intracellular Ca^{2+}. We here examine these responses in CHO-K1 cells expressing recombinant m1 (CHO-m1; 1000-2000 fmol of receptor / mg protein), m2 (CHO-m2; 75-125 fmol / mg protein), m3 (CHO-m3; 1000-2000 fmol / mg protein) (Buckley *et al*, 1989) or lower expressed m3 receptors (CHO-vt9; 200-300 fmol / mg protein) detected by [^{3}H] N-methyl scopolamine. Carbachol-stimulated Ins(1,4,5)P_3 accumulation was measured in these cell clones using a radioreceptor assay (Challiss *et al*, 1988). Carbachol dose response curves were constructed after 10 s incubation corresponding to the peak phase of Ins(1,4,5)P_3 accumulation. At the 10 s time point, peak responses were 384 ± 40 pmol /mg protein, 430 ± 30 pmol / mg protein and 224 ± 32 pmol / mg protein compared with basal values of 47 ± 12 pmol / mg protein, 33 ± 9 pmol / mg protein and 47 ± 12 pmol / mg protein in CHO-m1, CHO-m3 and CHO-vt9 cells, respectively. EC_{50} values (log molar) were -5.16 ± 0.06, -5.58 ± 0.12 and -4.49 ± 0.05 in CHO-m1, CHO-m3 and CHO-vt9 cells, respectively. All responses observed were pertussis toxin (PTX) insensitive (cells were pretreated with 100 ng / ml PTX for 20 h). Co-incubation of carbachol with CHO-m2 cells produced no increase in Ins(1,4,5)P_3 mass above basal levels.
Carbachol also stimulated Ins(1,4,5)P_3 accumulation in electroporated CHO-m1 cells displaying basal and maximal Ins(1,4,5)P_3 mass responses of 28 ± 7 pmol / mg protein and 120 ± 5 pmol / mg protein, respectively, at the peak 30 s time point with an EC_{50} (log value) of -5.46 ± 0.14.
$^{45}Ca^{2+}$-release from intracellular stores (Wojcikiewicz *et al*, 1990) of permeabilized CHO-m1, CHO-m3 and CHO-vt9 cells was also enhanced by carbachol. Whereas EC_{50} (log molar) values (-6.16 ± 0.04 and -6.11 ± 0.05 from CHO-m1 and CHO-m3 permeabilized preparations, respectively) and maximal release (70 %) of total loaded $^{45}Ca^{2+}$ were identical for m1 and m3 receptors, $^{45}Ca^{2+}$ release from permeabilized CHO-vt9 cells was substantially lower.(23 ± 7 % with an EC_{50} (log value) of -4.57 ± 0.14).
These results reveal that Ins(1,4,5)P_3 and Ca^{2+} release responses to muscarinic agonists are maintained in electrically permeabilized CHO cell clones and that m1 and m3 receptors expressed at a similar density produce identical phospholipase C activation and Ca^{2+} mobilization on exposure to carbachol. However, carbachol seemed to act as a less effective agonist in CHO-vt9 cells suggesting that this clone has too few m3 receptors to elicit a maximal response to carbachol.

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335P PROENDOTHELIN-1-INDUCED PHOSPHATIDYLINOSITOL TURNOVER IN RAT STRIATUM INVOLVES A PHOSPHORAMIDON-SENSITIVE ENDOTHELIN CONVERTING ENZYME

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Substantial evidence has accumulated indicating neuroendocrine actions for endothelin-1 (ET-1) (Stojilkovic & Catt, 1992). ET-1 and its receptors have been detected in neurones, glia and in the cerebrovascular system, but limited information is available concerning CNS production and metabolism of ET-1. In the periphery, the cleavage of a precursor (proET-1) is an obligatory event involving a specific endothelin-converting enzyme (ECE) (Masaki & Yanagisawa, 1992). Our goal was to detect such an ECE in the rat striatum using stimulation of phosphatidylinositol (PI) turnover as a marker of endothelin receptor stimulation by biologically active ET-1.

Male Sprague Dawley rats (200-300 g), were decapitated and their brains quickly removed. The striata were dissected free and cut into 0.35 mm thick slices using a McIlwain chopper. Slices were suspended in physiological buffer (in mM: NaCl 118, KCl 5.0, $CaCl_2$.1.3, KH_2PO_4 1.0, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 10) gently agitated, gassed with carbogen and maintained at 37°C. After 4 washes at 15 min intervals, the preparations were incubated for 30 min with 5 mM LiCl and 0.1 µM [^{3}H]*myo*-inositol. PI turnover was induced by proET-1 or ET-1, stopped 30 min later by addition of chloroform/methanol (1:2, v/v) and the amount of [^{3}H]-inositol monophosphates (IP1) generated measured using anion exchange chromatography and liquid scintillation counting (Richards, 1990).

ET-1 and proET-1 (from 0.001 to 3 µM) generated IP1 with similar kinetics, response ranges (from 77 ± 53 to 1675 ± 373 dpm and from 28 ± 17 to 1393 ± 243 dpm respectively) and EC_{50}s (100 ± 23 and 127 ± 37 nM respectively; n=8). These effects were not affected by inhibitors of aspartate (pepstatin), cysteine (p-hydroxymercuribenzoic acid) or serine proteases (leupeptin). Phosphoramidon completely inhibited responses to proET-1 (IC_{50} = 6.4 ± 1.1 µM; n=8) without affecting responses to ET-1. Thiorphan, an inhibitor of enkephalinase (EC 3.4.24.11), was about as potent as phosphoramidon at reducing the effects of proET-1 however, this probably does not reflect an ECE inhibitory activity alone since, at concentrations higher than 3 µM, thiorphan also markedly antagonised the effects of ET-1. Another enkephalinase inhibitor, (Des-Tyr^1)Met-enkephalin, did not significantly modify the proET-1-mediated response. Both thiorphan and (Des-Tyr^1)Met-enkephalin, at 1 and 3 µM, significantly augmented the ET-1-mediated effects by about 20% ($P < 0.05$), without modifying the EC_{50}. Such results suggest that, in the rat striatum, proET-1-mediated IP1-synthesis is dependant on an ECE activity similar to some already described in peripheral tissues (i.e. phophoramidon sensitive). The enkephalinase present in the tissue does not appear to possess any ECE activity generating biologically active ET-1, but is probably able to metabolise ET-1.

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336P ARE G-PROTEIN BETA/GAMMA SUBUNITS RESPONSIBLE FOR THE STIMULATION OF INS(1,4,5)P_3 FORMATION BY µ OPIOIDS IN SH-SY5Y CELLS?

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We have recently reported that mu, but not delta, opioids cause a transient (peaks at 15s) stimulation of Ins(1,4,5)P_3 formation in SH-SY5Y cells, mediated by a pertussis toxin sensitive G-protein (Smart et al., 1993). One possible mechanism for this effect is that beta/gamma subunits, liberated from G_i, could activate phospholipase C (PLC) (Birnbaumer, 1992). However, whilst both mu and delta opioid receptors are coupled via G_i to adenylyl cyclase (AC), there are more mu than delta receptors on SH-SY5Y cells (Yu et al., 1986). SH-SY5Y cells also possess $alpha_2$ adrenoceptors coupled via G_i to AC (Lambert & Nahorski, 1990). In this study we increased the intracellular concentration of beta/gamma subunits from G_i by selective activation of mu, delta and $alpha_2$ receptors. Whole cell suspensions (final volume 0.3ml) were preincubated at 37°C for 15min, and then incubated with or without [D-Ala^2,MePhe^4,Gly(ol)^5]enkephalin (DAMGO, 1µM), [D-Pen^{2,5}]enkephalin (DPDPE, 10µM) or clonidine (1µM) in various combinations for 15s. Ins(1,4,5)P_3 mass was measured using a specific radio-receptor assay (Challiss et al, 1988). Activation of the mu receptor with DAMGO stimulated Ins(1,4,5)P_3 formation in SH-SY5Y cells. However, DPDPE and clonidine, alone or in combination, had no effect on Ins(1,4,5)P3 formation (Table 1). These data strongly suggest that beta/gamma subunit activation of PLC is not the mechanism by which mu-opioids stimulate Ins(1,4,5)P_3 formation.

Table 1. The effects of DAMGO, DPDPE and clonidine on Ins(1,4,5)P_3 formation in SH-SY5Y cells.

Addition	Receptor Specificity	Ins(1,4,5)P_3(pmol/mg protein)
None		8.2 ± 0.4
DPDPE (10µM)	delta	9.3 ± 0.6
Clonidine (1µM)	$alpha_2$	7.3 ± 0.6
DPDPE + Clonidine	delta+$alpha_2$	8.9 ± 0.7
DAMGO (1µM)	mu	27.7 ± 1.0*
DAMGO + DPDPE	mu+delta	27.3 ± 1.1*
DAMGO + Clonidine	mu+$alpha_2$	27.4 ± 0.7*

(Mean ± SEM, where n=5. * denotes P<0.05 (t-test) increased compared to basal).

Financial support by Leicestershire Health Authority is gratefully acknowledged.

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337P OPIOID-INDUCED EMESIS IN THE FERRET

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Nausea and vomiting are common side effects of opioid therapy. Morphine is the prototypic μ opioid receptor agonist but it also interacts with κ and ∂ opioid receptors. The relative importance of each opioid receptor class in morphine-induced emesis is uncertain. The study of the emetic effects of opioids has been complicated by the limited selectivity of agonists and antagonists available and by variations in their degree of brain penetration.

Adult ferrets (0.6-1.0 kg) were pretreated with s.c. saline or opioid antagonists 30min before s.c. agonist. Animals (n= 4 or 5) were observed for 135 min and emetic activity was recorded as the number of retches and vomits occurring in 5 min time bins. The lowest dose of morphine which caused emesis in all animals tested was 0.5 mg/kg (median retches=20(range 4-52); median vomits=5(range 0-10). The κ receptor agonist ICI 204448 (Shaw et al., 1989) was dose-dependently emetic in all animals (0.1-5.0 mg/kg) At 0.25mg/kg median retches=27(2-38); median vomits=6(3-7). The ∂ receptor agonists leu enkephalin (0.05-5.0mg/kg), and [D-Pen^{2,5}]-enkephalin (0.1-5.0mg/kg) were not emetic. Pretreatment with the κ receptor antagonist norbinaltorphimine, 1.25 mg/kg 3 days earlier, abolished emesis due to 0.25mg/kg ICI 204448 ($p<0.05$ Mann Whitney) but had no effect on that due to 0.5 mg/kg morphine (median retches=17(2-23); median vomits=4(2-9), $p>0.1$). Pretreatment with the μ receptor antagonist naloxonazine 1.0 mg/kg abolished morphine-induced emesis ($p<0.05$) but had no effect on that due to 0.25 mg/kg ICI 204448 (median retches=29(12-33), median vomits=7(4-7), $p>0.1$). Pretreatment with the ∂ antagonist naltrindole 1mg/kg, had no effect on emesis due to 0.25 mg/kg ICI 204448 (median retches=27(21-36), median vomits=5(2-7), $p>0.1$).

These data suggest that opioids can elicit emesis through an action on μ and κ receptors. The differential effects of opioid receptor antagonists against morphine indicate that morphine-induced emesis is mediated through μ but not κ receptors. The role of ∂ receptors is less clear. ∂ agonists were not emetic, whereas the ∂ antagonist naltrindole inhibits morphine-induced emesis (Rudd & Naylor, 1992). One possible explanation is that the μ receptor involved is a μ-∂ complexed receptor. A wider range of ∂ agonists and antagonists is being assessed to clarify this.

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338P CHRONIC HEROIN/MORPHINE ADMINISTRATION DECREASES PROTEIN KINASE C IMMUNOREACTIVITY IN THE HUMAN AND RAT BRAIN

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The chronic administration of opiates has been shown to result in a marked sensitization of the adenylyl cyclase/cAMP system at every biochemical step between opioid receptors and physiological responses (i. e. increased cAMP production, abundance of G proteins and protein kinase A activity) (Nestler, 1992). To date the up-regulation of this transduction system has been viewed as the most relevant compensatory homeostatic response to the sustained inhibition of the opioid receptor system (tolerant/ dependent state) (Collier, 1980; Nestler, 1992). Recently, opioid receptors also have been shown to be coupled to phospholipase D and protein kinase C (PKC) activation (Chen & Huang, 1991; Mangoura & Dawson, 1993) which opens up new perspectives on the role of other signal transduction systems in opiate addiction. The aim of this study was to quantitate directly in the human brain the levels of PKC during heroin addiction. Specimens of human brain (frontal cortex-Brodmann's area 9) were obtained from 15 subjects who had died by opiate overdose or violent causes. The group of heroin addicts, with known history of opiate abuse, consisted of 8 men (age: 27±2 years, postmortem delay: 34±3 h). The control group consisted of 5 men and 2 women (age: 32±3 years, postmortem delay: 35±4 h) without a history of drug abuse. Solubilized PKC was separated by gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes (western blotting), labelled with a specific antibody (monoclonal anti-PKC, clone MC 5, Amersham), and quantitated by image analysis after enhanced chemiluminescence (Integrated Optical Density, IOD units) similarly as described for other proteins (Escriba et al., 1994) with modifications. Postmortem delays up to 48 h did not affect the quantitation of PKC. In the brain of heroin addicts the immunoreactivity of PKC (20.3±4.9 IOD units) was markedly decreased (53%, $P< 0.05$) as compared with that in control subjects (43.2±10.1 IOD units). The decrease of PKC in the brain of human addicts paralleled that observed in the cerebral cortex of rats after repeated treatment with morphine (10 to 100 mg/kg i.p. for 5 days) (saline, PKC: 25.9±1.9 IOD units, n=8; morphine, PKC: 17.1±0.7 IOD units, n=6, decrease: 34%, $P<0.05$). Acute treatment with morphine (10 mg/kg i.p. for 2 h) did not alter the expression of PKC in the rat brain (PKC: 24.8±1.6 IOD units, n=8). In morphine-dependent rats (5 days), naloxone (2 mg/kg i.p. for 2 h)-precipitated withdrawal, induced a rapid and strong behavioural reaction with a concomitant up-regulation of PKC immunoreactivity to control values (PKC: 27.5±2.6 IOD units, n=5). The data demonstrate the induction of down-regulation of PKC in opiate addiction by a naloxone-sensitive mechanism, which suggests that PKC plays a role in the signal transduction pathway of μ-(and probably other)-opioid receptors. These results could help to explain the molecular processes of opiate tolerance, dependence and abstinence syndrome.

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Supported by FIS (Project 93/0641) and CITRAN (Project 6/1992).

339P METOPRINE AND NOCICEPTION IN THE MOUSE

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Metoprine, an inhibitor of histamine N methyltransferase, the major enzyme of central histamine metabolism, has been shown to increase brain histamine levels in rats (Duch *et al*, 1978) and to cause a fall in body temperature in mice (Sturman *et al*, 1991). This hypothermia may alter tail-skin temperatures which in turn affect latencies in the tail-flick test (Han & Ren, 1991). The present study was designed to investigate antinociceptive effects of metoprine in a variety of procedures, including the mouse tail immersion test, where a thermal stimulus is also applied to the tail.

Groups of at least 8 female BK:TO mice (20-35g) were treated with 0-20 $mg.kg^{-1}$ metoprine i.p and tested in either the tail immersion (50°C), hot-plate (55°C) or abdominal constriction test (induced by 0.6% acetic acid i.p.). Tail-skin temperatures were measured using a Comark electronic thermometer.

A partial negative correlation between tail-skin temperature and tail immersion latency was found (latency = 14.3 - 0.249 x tail-skin temp, $r = 0.506$, d.f.= 37, $p<0.002$). Metoprine (20 $mg.kg^{-1}$) caused a decrease in tail-skin temperatures (-0.4±0.3°C) when compared with pre-dosing temperatures which was significantly different ($p<0.05$; ANOVA) from that caused by administration of vehicle (+0.4±0.2°C). However, there was no difference in tail immersion latencies between control and metoprine-treated groups, neither did this treatment alter latencies on the hot-plate, another test affected by changes in body temperature (Freeman *et. al.*,1992). However, metoprine (20 $mg.kg^{-1}$ but not 10 $mg.kg^{-1}$) completely abolished abdominal constrictions induced by 0.6% acetic acid compared with the control group measured 30 min after administration (0 (0-0) and 9 (0.5-23) [medians and inter-quartile ranges], $p<0.01$; Mann-Whitney). This blockade was not reversed by pretreatment of the mice with naloxone (5 $mg.kg^{-1}$ s.c.).

Thus, metoprine had no effect in the two thermal models used here, despite causing a fall in tail-skin temperature, but caused antinociception via a non-opioid mechanism in a chemical test. This may be unrelated to its action in raising brain histamine levels.

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340P INTRATHECAL ADMINISTRATION OF LAMOTRIGINE INDUCES ANALGESIA IN ACUTE AND CHRONIC PAIN MODELS IN RATS

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Lamotrigine (Lamictal®), a new antiepileptic drug, has been shown to produce analgesia when given orally in an acute model of prostaglandin E_2-induced hyperalgesia in rats (Nakamura-Craig and Follenfant, 1992a) and in a neuropathic pain model in diabetic rats (Nakamura-Craig and Follenfant, 1992b). We have now investigated the effects of intrathecal administration of lamotrigine in these models. Intrathecal (IT) cannulation was performed in male Wistar-strain rats (300-400g) under general anaesthesia with pentobarbital sodium (60 $mgkg^{-1}$, i.p.) using a modified technique described by Yaksh and Rudy (1976). The drugs were injected intrathecally in 10 µl followed by 4 µl of saline, 6-7 days after the IT cannulation. Acute hyperalgesia was induced by subplantar injection of prostaglandin E_2 (PGE_2, 100 ng). Chronic hyperalgesia was determined in diabetic rats 3 to 4 weeks after treatment with streptozotocin (70 $mgkg^{-1}$, i.p.). Paw withdrawal response (reaction time, s) to paw pressure (40 mmHg, constant) was measured using a modification of the Randall-Selitto test (Randall and Selitto, 1975) in both acute and chronic models of hyperalgesia. Data is presented as % of analgesia calculated for individual animals in each treatment group using the formula $\frac{\Delta \text{ reaction time}}{\text{mean } \Delta \text{ reaction time of control animals}} \times 100$, where

Δ reaction time was obtained by subtracting the value measured after administration of substances from the pretreatment reaction time. Intrathecal injections of lamotrigine (12.5, 25 and 100µg) given to rats 150 min after PGE_2 subplantar injections induced dose-dependent analgesia (37±4%, 52±9% and 95±5% of analgesia, mean±s.e.m.; n≥5; $P<0.05$, respectively) 30 min after the injection and was still present at least 5 h later (34±3%, 50±11% and 89±7% of analgesia, respectively) without causing any motor impairment. In this model, morphine (2.5 µg) induced potent analgesia 30 min after the injection (99±3% of analgesia, $P<0.05$) but the effect was reduced 5 h later (54±3% of analgesia, $P<0.05$). Lamotrigine (12.5 and 25 µg) administered intrathecally to diabetic rats also induced a dose-dependent analgesia (56±7% and 83±11% of analgesia, respectively, $P<0.05$) 30 min after the injection. This effect was still present at least 24h after the administration (33±12% and 81±4% of analgesia, $P<0.05$, respectively). Intrathecal administration of morphine (2.5µg) to diabetic rats induced maximal analgesia 30 min after the injection but no analgesic effect was observed 24 h later. By contrast, the antiepileptic drug carbamazepine (100µg) administered intrathecally did not induce significant analgesia in both acute and chronic model of pain. Lamotrigine therefore induced a long lasting spinal antinociceptive effect. Thus lamotrigine, given by the epidural route may be useful for the treatment of some chronic pain conditions.

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341P THE EFFECT OF HISTAMINE H_2 RECEPTOR AGONIST AND ANTAGONIST ON RESTRAINT-INDUCED ANTINOCICEPTION IN FEMALE MICE IS DEPENDENT ON THE TIME OF DRUG ADMINISTRATION

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Previous studies have shown that histamine H_2 receptors are involved in physical dependence on and tolerance to opioids (Wong & Roberts, 1975), as well as swim-induced analgesia in mice (Wong, 1987; Oluyomi & Hart, 1991). With respect to stress-induced analgesia, there is some preliminary evidence to suggest that histamine H_2 receptor agonists and antagonists may act by altering the intensity of stress thus affecting the antinociceptive effect observed (Wong, 1993). The present work was undertaken to study whether or not the effect of histamine H_2 receptor agonists and antagonists is dependent on the time of administration in relation to restraint in female mice.

Female CFLP mice weighing between 20-25 g were used. The animals were restrained individually in a cylindrical tube with holes along the whole length for ventilation (Wong & Bentley, 1979). Each animal would have only a limited degree of movement inside the tube. The animals were restrained for one hour then tested for latency response to hot plate test at 55°C, at 0, 10, 20, 40 and 60 min post-restraint. The histamine H_2 receptor agonist, dimaprit, and antagonists, cimetidine and zolantidine dimaleate, were given s.c. either 15 min before restraint or immediately after restraint for one hour. Control animals received saline injection 10 ml kg^{-1} at the corresponding time. At least 16 animals were used for each treatment. Mean hot plate response times were compared with the analysis of variance.

Restraint for 1 h induced significant antinociceptive activity in female mice (10.21 ± 0.77 s versus 5.02 ± 0.33 s in controls, $P<0.01$). The antinociceptive activity was significant throughout the 1 h period of observation. Prior administration of dimaprit (1.5-6.0 mg kg^{-1}) further enhanced the antinociceptive activity; peak antinociceptive activity was 13.85 ± 0.63 s versus 10.21 ± 0.77 s in saline restraint control ($P<0.01$). Furthermore, the induction of antinociception was antagonised by prior administration of cimetidine (2.5-10.0 mg kg^{-1}) or zolantidine (2.5-10.0 mg kg^{-1}). With these treatments, peak antinociceptive activity was reduced to 8.12 ± 0.31 s ($P<0.05$) and 6.31 ± 0.30 s ($P<0.01$) respectively. However, when these drugs were administered immediately after restraint for 1 h, the antinociceptive activity observed was similar to those restrained animals receiving saline injection. The histamine receptor agonist and antagonists, at the doses used in the present study, did not affect the response of unrestrained animals to the hot plate test. These results demonstrate that the effect of histamine H_2 receptor agonist and antagonists on restraint-induced analgesia is dependent upon their time of administration and may act by altering the intensity of stress, thus affecting the antinociceptive activity induced.

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342P LAPAROTOMY IN SHEEP INDUCES A REDUCTION IN THRESHOLD RESPONSES TO NOXIOUS THERMAL STIMULATION, BUT NOT TO NOXIOUS MECHANICAL STIMULATION

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Reduction in threshold responses to noxious thermal and mechanical stimulation has been recorded in laboratory animals following acute and chronic inflammatory stimulants and nerve trunk section. These alterations in threshold responses are considered to be a result of alterations in both peripheral nociceptor function and central neuronal activity (Woolf, 1983). Whether or not this occurs clinically in response to acute surgical trauma is not clearly established. This present study attempted to identify alterations in responses to noxious stimulation in sheep undergoing surgery.

Eighteen Scottish Blackface ewes undergoing a midline laparotomy for collection of 4 day old embryos under general anaesthesia, were divided randomly into 3 groups of 6 animals. Four similar animals (group 4) undergoing anaesthesia, but not surgery, were also used. Threshold responses to noxious mechanical and thermal stimulation were recorded according to the methods of Nolan et al. (1987) over a 30 min period before induction of anaesthesia, and at intervals for 2 hours after anaesthesia ended. General anaesthesia was induced with thiopentone sodium, 18-20 mg/kg, and the trachea intubated. Anaesthesia was maintained with halothane in a nitrous oxide (50%)/oxygen (50%) mixture. Immediately after embryo retrieval, groups 2 and 3 received the non-steroidal anti-inflammatory drug (NSAID), flunixin meglumine, 1mg/kg, intravenously (i.v.) or the opioid, buprenorphine, 10 mg/kg, i.v., respectively. Group 1 received no intraoperative treatment. Results were analysed using a one way analysis of variance (pre vs 2h) and a General Linear Model routine. Differences were judged to be significant where $p \leq 0.05$.

Mean pre-anaesthesia thresholds to noxious thermal and mechanical stimulation were 58.8, 57.7, 56.5 and 52.7°C, 6.6, 6.5, 5.7 and 7.1N, for groups 1-4 respectively. There were no significant differences between these values. The mean duration of anaesthesia was 22 min for all groups. There was a significant reduction in the response to thermal stimulation after surgery for sheep in group 1 (45[49.7 ± 1.66], 60 [51.4 ± 1.72] and 120 min [54.9]) , while responses to mechanical stimulation remained unaltered. No differences were recorded at any time points in groups 2 and 3, when stimulation remained unaltered. No differences were recorded at any time points in groups 2 and 3, when compared with pre-anaesthesia/surgery values. Responses to thermal and mechanical stimulation in sheep undergoing anaesthesia but not surgery (group 4) were unaltered during the 2 hour recording period after anaesthesia ended.

These results indicate that laparotomy in sheep induces the development of a reduction in the threshold to noxious thermal stimulation, but not to noxious mechanical stimulation. It is considered likely that this reflects an alteration in central neuronal function, as the site of thermal stimulation, the media aspect of the ear pinna, was far removed from the site of injury. This effect was not recorded in animals receiving either, flunixin meglumine or buprenorphine given after the initial surgical incision (at 45 min post-op, 57.1 ± 2.42 and 56.7 ± 1.57 respectively). Both drugs have been shown to be analgesic in sheep (Nolan et al., Chambers et al., 1993), although neither drug induced analgesia in the present study. However, both appeared to prevent the development of post-operative thermal hyperalgesia.

This work was supported by the Agriculture and Food Research Council. We wish to acknowledge the help of the staff at IAPGR, Roslin.

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343P TOURNIQUET APPLICATION INDUCES ALLODYNIA TO NOXIOUS MECHANICAL STIMULATION IN SHEEP

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Tourniquet (Tq) pain has been used as a model of ischaemic pain in animals. It has been shown that post-ischaemic/reperfusion hyperalgesia can be abolished by pre-treatment of rats with the non-steroidal anti-inflammatory drug (NSAID), lysine acetylsalicylate, although the latency to aversion is not altered (Gelgor et al., 1986). Recent work has suggested that 'central sensitization' may be important in the development of reperfusion hyperalgesia (Gelgor et al., 1992; Sher et al., 1992). However, peripheral involvement cannot be excluded. Consequently, we decided to study this further.

Thresholds to noxious mechanical stimulation were recorded from 5 adult sheep as previously described (Nolan et al., 1987). Subsequently, a pneumatic Tq was applied proximal to the carpus and inflated to a pressure of 300mm Hg until signs of aversion were recorded, or for a maximum period of 30 min. Sheep received either no treatment (group 1), saline, 0.9%, 5 ml (group 2) or the NSAID, flunixin meglumine, 1 mg/kg (group 3) intravenously, 60 min before Tq inflation or fentanyl (group 4), 5 μg/kg i.v. 5 min before inflation of the Tq. Thresholds to mechanical stimulation were recorded at 5 min intervals during the period of Tq inflation from the ipsilateral limb (IL) (groups 1,2,3,4) and the contralateral limb (CL) (group 1 only) and until thresholds returned to normal after deflation. Time to aversion (TA) was also recorded Oneway analysis of variance was used to determine variation between treatment groups. Differences within groups were evaluated using the Student's t test. A Kruskal Wallis test for non-parametric data was used to test time to aversion data. $p<0.05$ was considered significant. Thresholds to mechanical stimulation dropped from 3.1 ± 0.3N to 1.7 ± 0.3N within 15 min Tq inflation ($p<0.01$) on the IL (group 1), but remained unchanged on the CL (3.6 ± 0.6N vs 3.4 ± 0.8N). Saline treatment induced a similar response, control thresholds 2.8 ± 0.2N vs 1.5 ± 0.2N at 15 min ($p<0.01$). There were no significant differences between the last thresholds recorded before aversion (2.1 ± 0.6N) and control values (2.8 ± 0.2N) in sheep treated with flunixin meglumine. Sheep that received fentanyl showed a significant increase in thresholds 5 and 10 min after Tq inflation (control, 3.4 ± 0.3N; 5 min 6.6 ± 0.9, $p<0.01$). Threshold responses returned to control values in all groups within 5 min of deflating the Tq, although in sheep that received fentanyl, the mean value (5.5N) was greater than control values (3.4N) ($p=0.07$). The mean TA was not different between groups 1,2,3 and 4 (20.6 ± 0.5, 22.6 ± 0.5, 25.2 ± 1.2 and 27.0 ± 2.7 min respectively).

While post-ischaemic hyperalgesia to noxious mechanical stimulation was not recorded, hyperalgesia to mechanical stimulation was present during inflation of the Tq on the IL but not the CL, suggesting that this effect has a strong peripheral component. This has not been demonstrated previously, possibly because workers have focused on events after deflation of the Tq. The absence of reperfusion hyperalgesia may be due to the test system used although Sher and Mitchell (1990) reported the development of mechanical hyperalgesia in a model of hindlimb ischaemia induced by acute occlusion of the femoral artery. Pre-treatment with a NSAID abolished the hyperalgesia, but did not alter the TA indicating that the mechanisms of Tq-induced pain and Tq-induced hyperalgesia are different.

ACKNOWLEDGEMENT: This work was supported by the Agricultural Food Research Council.

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344P CHARACTERISATION OF THE SPECIFIC BINDING OF THE HISTAMINE H_3 RECEPTOR ANTAGONIST RADIOLIGAND [^{3}H]GR168320

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Histamine H_3 receptor binding sites were originally identified in the CNS using the histamine H_3 agonist radioligands [^{3}H](R)-α-methylhistamine (RAMH; Kilpatrick et al.,1992) and [^{3}H]N$^{\alpha}$-methylhistamine (NAMH; West et al., 1990). We now describe the characteristics of a new histamine H_3 receptor antagonist radioligand [^{3}H]GR168320 ([3,4-$^3H_2$]-Cyclohexyl-{[4-(3H-imidazol-4-yl)-piperidin-1-yl]-imio-methyl}-amine).

Rat cerebral cortices were homogenised and washed three times using HEPES buffer (50 mM, pH 7.4) with centrifugation. The homogenate (0.8-0.9 mg protein per assay tube) was incubated with [^{3}H]GR168320 (4.8 Ci/mmol, 0.1 nM) in an assay volume of 5 ml for 30 min (37°C). Reactions were terminated by filtration and washing with cold buffer (3x5 ml). Non-specific binding was defined by the inclusion of thioperamide (10 μM). All data are expressed as mean values ± s.e. mean (n=3). Under these conditions, specific binding was routinely 80-90% of the total binding. Saturation studies with [^{3}H]GR168320 (0.02-10 nM) revealed a single saturable site of high affinity (B_{max} 412±89 fmol/mg protein; K_d 0.11±0.01 nM). Specific binding associated and dissociated rapidly (k_{+1}=3.00±0.61x10^7 M^{-1}s^{-1}; k_{-1}=1.25±0.31x10^{-2} s^{-1}). In competition experiments, the H_3 receptor agonists and antagonists potently inhibited binding (Table). In contrast, the histamine H_1 and H_2 receptor antagonists mepyramine and ranitidine weakly inhibited binding (pIC_{50} 4.58±0.10 and 4.80±0.09 respectively). Hill slopes (nH) for H_3 antagonists were close to unity while those of H_3 agonists were routinely less than 1.

Table. Affinity estimates (pIC_{50}) and nH of H_3 antagonists and agonists to inhibit specific [^{3}H]GR168320 binding to rat cerebral cortices.

Antagonist	pIC_{50}	nH	Agonist	pIC_{50}	nH
Thioperamide	7.97±0.07	1.11±0.05	Histamine	7.18±0.12	0.67±0.07
VUF9153*	9.16±0.06	1.05±0.06	NAMH	7.80±0.07	0.71±0.01
GR168320	9.32±0.02	1.07±0.02	RAMH	7.93±0.02	0.72±0.05

*1-(4-Chloro-benzyl)-2-[3-(1H-imidazol-4-yl)-propyl]-isothiourea

We conclude that [^{3}H]GR168320 specifically labels histamine H_3 receptors in homogenates of rat cortex and provides a useful tool for the study of histamine H_3 receptors. The affinity estimate for thioperamide correlates well with that obtained by Kilpatrick et al., (1992), although affinity estimates for the H_3 agonists are lower. These differences may be due to the shallow Hill slopes for the agonists obtained with [^{3}H]GR168320 and experiments are being carried out to investigate this further.

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345P INFLUENCE OF SODIUM IONS ON THE BINDING OF THIOPERAMIDE TO HISTAMINE H_3 RECEPTORS IN RAT CEREBRAL CORTEX

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Studies of the binding of ^{3}H-N-α-methylhistamine (^{3}H-N-αMeHA) to the histamine H_3-receptor in rat cerebral cortical membranes have indicated the presence of two separate high affinity binding sites for this ligand with differing affinities for the H_3-receptor selective antagonist thioperamide (West *et al.*,1990). However, Arrang *et al.*,(1987,1990) found no evidence for heterogeneity of thioperamide binding in this tissue under different conditions. In this communication we report that the presence of sodium ions can significantly alter the binding characteristics of thioperamide, but not other H_3-ligands, to the histamine H_3-receptor in rat cerebral cortex.

Binding studies were performed in homogenates of rat (male lister hooded rats) cerebral cortices in 50mM Tris buffer (plus 0, 50 or 100mM NaCl) pH=7.4 containing 1nM ^{3}H-N-αMeHA (60min,37°C) essentially as described by West *et al.*,(1990). Non-specific binding was defined with 10 μM thioperamide. The histamine H_3-receptor agonists N-αMeHA & R-α-methylhistamine and the H_3-receptor antagonists thioperamide, clobenpropit (Van der Goot,*et al.*,1992) & SKF 91486b (Arrang *et al.*,1990) potently inhibited specific ^{3}H-N-αMeHA binding (IC_{50}'s = 4.8±0.8, 7.6±0.8, 64.6±3.2, 1.6±0.2, 15.7±3.2 nM respectively, n=3-6). Competition curves fitted well to a single site (i.e.Hill coefficients (nH) not significantly different from unity) with the exception of thioperamide (Table) which fitted best to a two component model ($IC_{50(1)}$ 0.6nM, $IC_{50(2)}$ 68.9nM, % site (1)= 20%). Addition of 50 or 100mM NaCl, decreased the specific binding of 1nM ^{3}H-N-αMeHA by 41.7±1.3 & 50.7±2.3%, without altering the competition binding parameters of the H_3-agonist N-αMeHA and H_3-antagonist clobenpropit (Table). However, the presence of sodium ions shifted the competition curve for thioperamide to the left and significantly increased the Hill coefficient (Table).

Table. IC_{50} values mean±s.e.mean (nM, n=3) for inhibition of ^{3}H-N-αMeHA (1nM) binding.

	0mM Na^+	50mM Na^+	100mM Na^+
N-αMeHA	6.2 ± 0.2	5.52 ± 0.02	7.7 ± 0.3
Clobenpropit	1.3 ± 0.2	1.10 ± 0.10	1.5 ± 0.2
Thioperamide	64.9 ± 4.5*	8.40 ± 0.70	7.1 ± 0.4

* nH (-0.73±0.03) significantly different from unity.

These results indicate that the composition of the incubation medium is an important factor in determining the binding characteristics of thioperamide. However, it is yet unknown whether this difference is due to the presence of two separate high affinity binding sites or as a result of a conformational change at a single site.

Supported by MRC and Glaxo Group Research.

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346P ONTOGENESIS OF NPY CONTENT, NPY RECEPTORS AND G-PROTEINS IN CEREBRAL CORTEX OF NORMOTENSIVE AND HYPERTENSIVE RATS

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We have studied the ontogenesis of NPY content, NPY receptors, and α-subunits of the G-proteins G_i and G_o in cerebral cortex of male normotensive (WKY) and spontaneously hypertensive rats (SHR) at the ages of 3, 6, 8 and 28 weeks and of female WKY and SHR at 28 weeks. NPY content was determined by radioimmunoassay (Rascher et al. 1993). NPY receptors were quantified by [^{125}I]NPY saturation binding (Feth et al. 1991). G-protein α-subunits were measured by quantitative immunoblotting using the specific antisera AS/7 ($G_{i\alpha}$) and GC/2 ($G_{o\alpha}$); pertussis toxin-catalyzed ADP-ribosylation was also quantified (Michel-Reher et al. 1993). Data are mean ± s.e.mean of 6-11 rats.

NPY content was ≈400 pmol/g wet weight in WKY and showed no major differences between age groups and sexes. In SHR NPY content was reduced by ≈20-40% compared to age- and sex-matched WKY; this was most pronounced in the youngest age groups and failed to reach statistical significance in adult rats. During ontogenesis NPY receptor density increased from 94±9 in 3 week to 178±29 fmol/mg protein in 28 week old male WKY, but was only 105± 12 fmol/mg protein in female WKY. NPY receptor density in SHR was not significantly different from age- and sex-matched WKY. NPY receptor affinity for [^{125}I]NPY was similar in all male groups irrespective of age and strain (K_d ≈400 pM) but higher in the female groups (K_d ≈170 pM). Pertussis toxin substrates were 8.7±0.7 and 14.6±1.5 pmol/mg protein, respectively, in 3 and 28 week old male WKY. Immunoblotting showed that this was due to elevated $G_{o\alpha}$ and $G_{i\alpha}$. G-protein α-subunit abundance did not significantly differ between sexes or strains.

We conclude that age, sex and spontaneous hypertension regulate components of the NPY, NPY receptor, $G_{o\alpha}/G_{i\alpha}$ system in a differential manner. The physiological consequences of these alterations remain to be assessed in functional tests.

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347P AFFINITY PURIFICATION OF SYNAPTOSOMES USING A BIOTINYLATED DERIVATIVE OF ω-CONOTOXIN GVIA

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(Introduced by D. Lodge)

ω-Conotoxin GVIA (ω-CgTx GVIA), an irreversible blocker of N-type voltage dependent calcium channels (VDCCs), is a potent and selective inhibitor of Ca^{2+} entry into chicken forebrain synaptosomes (Bowman et al., 1993; Lundy et al., 1991). In contrast, Ca^{2+} entry into rat cortical synaptosomes is only partially blocked by the toxin (Bowman et al.,1993; Lundy et al., 1991) which suggests that N-type VDCCs comprise only a small subset of channels in this preparation. However, synaptosome preparations represent a heterogeneous population of neurotransmitter specific nerve terminals and it may be that only a minority of rat cortical synaptosomes possess ω-CgTx GVIA-sensitive N-type VDCCs.

To investigate this, we have developed a method for the affinity purification of synaptosomes expressing ω-CgTx GVIA-sensitive, N-type VDCCs. The method utilises a biotinylated derivative of ω-CgTx GVIA which retains its ability to displace ^{125}I ω-CgTx GVIA from its binding sites on rat synaptic membranes. This biotinylated ω-CgTx GVIA has been coupled to magnetisable beads and used successfully to bind deaggregated synaptosomes. The effect of ω-CgTx GVIA on KCl -induced increase in free intracellular Ca^{2+} ($[Ca^{2+}]_i$) elevation in parent, bound and unbound fractions of chicken and rat synaptosomes was investigated by using fura-2 based fluorimetry. In the chicken synaptosome parent fraction, ω-CgTx GVIA (0.1nM-1μM) produced a concentration-dependent block of $[Ca2+]_i$ elevation with an IC50 value of 28nM. All synaptosomes appeared to be captured by the affinity ligand because no increase in$[Ca2+]_i$ elevation was observed in the unbound fraction when challenged with KCl. Analysis of the affinity purified fraction by electron microscopy clearly shows the presence of single synaptosome-like structures attached to the beads. ω-CgTx GVIA (1μM) blocked only 23±5% of the $[Ca2+]_i$ elevation in the rat synaptosome parent fraction. Although, not all synaptosomes were captured by the beads, the block of free $[Ca2+]_i$ elevation in the unbound fraction by ω-CgTx GVIA (1μM) was reduced to 11±4%. No increase in $[Ca2+]_i$ elevation was observed in the affinity purified fraction, but synaptosome-like structures were clearly visible at the electron microscope level.

We conclude that ω-CgTx GVIA-sensitive N-type calcium channels are present on all chicken forebrain synaptosomes but only a minority of rat cortical synaptosomes.

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348P 7-NITRO INDAZOLE UNCOUPLES CEREBRAL BLOOD FLOW FROM METABOLIC DEMAND IN THE CONSCIOUS RAT

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Inhibition of nitric oxide synthesis (NOS) with 7-nitro indazole (7-NI) produces antinociceptive effects in mice but unlike N^G-substituted arginine analogues does not increase mean arterial blood pressure (MABP) *(Moore et al., 1993)*. It has therefore been suggested that 7-NI might not affect vascular endothelial cell NOS activity. In the present study, we have examined the effects of 7-NI upon aspects of both cardiovascular function, and cerebrovascular perfusion in the neocortex of conscious rats.

Conscious, lightly restrained adult rats were injected (i.p.) with either 25mg/kg (n = 10) or 50mg/kg (n = 10) of 7-NI in sesame seed oil, or with vehicle alone (n = 10). Equal numbers of treated and control rats were used for the measurement of local cerebral blood flow (LCBF) or local cerebral glucose utilization (LCGU) using the fully quantitative [^{14}C]-iodoantipyrine *(Sakurada et al., 1978)* and [^{14}C]-2-deoxyglucose *(Sokoloff et al., 1977)* quantitative autoradiographic techniques respectively. Arterial blood pressure and heart rate were monitored continuously. Data were analysed using Scheffe's test with significance set at $P < 0.05$, and are presented as mean ± s.e.mean.

Neither dose of 7-NI had any effect upon MABP, but following 25mg/kg heart rate was significantly decreased from 390 ± 19 to 262 ± 6 beats/min (-33%) with a similar effect at 50mg/kg (-35%). Despite the apparent lack of peripheral vasoconstriction, dose-dependant decreases in neocortical LCBF (ml/100g/min) indicated cerebrovascular constriction. At 25mg/kg these decreases were significant in all but frontoparietal cortex, but were most marked (>40%) in piriform (from 129 ± 4 to 74 ± 9) and occipital cortex (from 200 ± 19 to 111 ± 9). Further significant decreases were found following 50mg/kg, including now frontoparietal cortex (from 189 ± 18 to 110 ± 11). Significant decreases in LCGU (μmol/100g/min) were evident only at 50mg/kg, in cingulate (from 94 ± 3 to 75 ± 2), parietal (from 74 ± 3 to 63 ± 2) and entorhinal cortex (from 47 ± 2 to 38 ± 1), but at both doses, and in all cortical regions, the ratio of LCBF to LCGU decreased following 7-NI. Dose-dependent decreases in the ratio (indicative of blood flow uncoupled from metabolic demand) were most marked in piriform (from 2.48 to 1.51 and 1.12 at the two doses) and occipital cortex (from 2.2 to 1.29 and 1.12).

This study confirms that 7-NI does not alter MABP in the conscious rat, but the effects upon heart rate indicate that the drug is not totally devoid of cardiovascular effects. Within the cerebrovasculature however, decreases in LCBF in the absence of similar changes in LCGU indicate a direct inhibitory effect upon endothelial NOS to induce vasoconstriction. In this respect, the effect of 7-NI is similar to that of the N^G-substituted arginine analogues.

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349P THE EFFECT OF DEPOLARISATION ON ARGININE UPTAKE BY CEREBELLAR AND CORTICAL SYNAPTOSOMES

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Previous studies in this laboratory have identified a synaptosomal high-affinity arginine transport system, the activity of which is enhanced by removing extracellular Na^+ (Aldridge & Collard 1993). One mechanism which might account for this observation is an increase in cytosolic Ca^{2+} caused by reducing plasma membrane Na^+/Ca^{2+} exchange (Collard et al. 1988). Since synaptosomal NO synthase is a Ca^{2+}-dependent enzyme (Meyer et al. 1992), it seemed relevant to determine whether the synaptosomal arginine carrier could also be activated in a Ca^{2+}-dependent manner by procedures other than removing extracellular Na^+. Using techniques similar to those employed in the previous study (Aldridge & Collard 1993), this study examined the effect of depolarisation with K^+ and 4-aminopyridine (4-AP) on [^{3}H]-L-arginine uptake into rat cerebellar and cortical synaptosomes. The results are shown in table 1.

Table 1. [^{3}H]-L-arginine uptake (pmol/mg protein/minute). Results are given as the mean ± s.e. mean of 4-6 observations. Significant differences between controls and treated tissues are given by *P<0.05, **P<0.02, ***P<0.01 (Student's t test).

	Control	4-AP 250uM	4-AP 500uM			
Cortex	101.01 ± 3.35	100.19 ± 4.58	100.93 ± 5.44			
Cerebellum	111.51 ± 4.95	121.07 ± 6.23	123.93 ± 5.31			
	Control	25mM K^+(-Ca^{2+})	25mM K^+(+Ca^{2+})	50mM K^+(-Ca^{2+})	50mM K^+(+Ca^{2+})	
Cortex	122.61 ± 9.62	88.79 ± 5.47**	82.87 ± 4.59***	82.72 ± 7.71**	75.32 ± 3.48***	
Cerebellum	122.96 ± 5.56	94.38 ± 4.75**	97.21 ± 7.58*	80.12 ± 7.88***	79.61 ± 14.12*	

The results show that 4-AP at concentrations which should significantly depolarise synaptosomes (Herrero et al 1992) had little effect on [^{3}H]-arginine uptake. Elevating extracellular K^+ inhibited uptake both in the presence and absence of extracellular Ca^{2+}. The failure of 4-AP to influence uptake suggests that the inhibitory effect of raising extracellular K^+ may not be related to the depolarising effect of this procedure, but to some other influence on the ionic requirements of the nerve terminal arginine carrier.

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350P NITRIC OXIDE INDUCES CHANGES IN MAP2 GENE EXPRESSION IN HIPPOCAMPAL GRANULE CELLS

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Long term potentiation of synaptic transmission in the hippocampus is generally mediated through the N-methyl-D aspartate (NMDA) receptor complex, and can induce sustained alterations in granule cell gene expression (Morris *et al.*, 1988). We recently reported that administration of nitric oxide (NO) onto the molecular layer of the dentate gyrus granule cells caused changes in gene expression similar to those seen after LTP (Johnston & Morris, 1993). We have now investigated whether administration of NMDA, 8Bromo-cyclic GMP (8BrcGMP) and Sin 1 molsidomine (Sin-1) can cause changes in the expression of the mRNA of the gene encoding microtubule-associated protein 2 (MAP2), a cytoskeletal protein, levels of which are known to increase during neurite outgrowth (Ferreira *et al.*, 1990). Male wistar rats (180-200g) were given a unilateral injection into the molecular layer of the dentate gyrus using standard stereotaxic techniques. All drug combinations were delivered in a total volume of 75nl physiological saline, given over a 3 min period. The animals were sacrificed 24 hours later and 20 μm cryostat sections of the brain were processed for in-situ hybridisation using a ^{35}S-dATP-labelled oligonucleotide probe specific for MAP2 mRNA (Garner & Matus, 1988). The hybridisation signal in the region of the injection was expressed as a percentage of the signal in the equivalent region on the contralateral side and significance was tested by ANOVA with post-hoc t-test. Whereas vehicle injection caused no change in the MAP2 signal (-6 ± 4.5% in cell bodies and 0 ± 7% in dendrites n = 8), NMDA (50μM) increased the signal in the soma by 13 ± 7% ($p < 0.05$) and the dendritic signal by 24 ± 6% ($p < 0.05$) n = 3. 8BrcGMP (500μM) increased the somatic signal by 35 ± 12% $p < 0.01$ and the dendritic signal by 35 ± 6% $p < 0.01$ n = 5. Sin-1(1mM) a releaser of NO, significantly increased the MAP2 signal in the soma (13 ± 2% $p < 0.05$ n = 3). These results suggest that stimulation of the NMDA receptor may cause sustained structural changes in the granule cells of the dentate gyrus by enhancing MAP2 synthesis. This could play a role in the enhanced sensitivity of these cells during LTP.

This work was supported by grants from the MRC and the Royal Society. We are grateful to Prof. J Garthwaite for the gift of sin-1 molsidomine.

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351P ISOFORM SELECTIVITY OF INDAZOLE-BASED NITRIC OXIDE SYNTHASE INHIBITORS

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7-nitro indazole (7-NI) is a potent inhibitor of rat and mouse cerebellar nitric oxide synthase (NOS) in vitro (Babbedge et al., 1993). Inhibition of NOS, most probably within the dorsal spinal cord, accounts for the antinociceptive effect of 7-NI in the mouse (Moore et al., 1993). The ability of 7-NI, 3-bromo 7-NI, 2,7 dinitro 7-NI and L-N^G nitro arginine (L-NOARG) to inhibit both constitutive (rat cerebellar, RC; bovine endothelial, BE) and induced rat lung (RL; following i.p. administration of E.coli endotoxin, 5 $mgkg^{-1}$, 6 h before sacrifice) NOS isoforms in vitro has been determined.

The preparation of rat cerebellar, bovine endothelial and rat lung homogenates and assessment of NOS enzyme activity by the conversion of radiolabelled L-arginine to radiolabelled citrulline are detailed elsewhere (Babbedge et al., 1993). 7-NI, 3-bromo 7-NI, 2,7 dinitro 7-NI and L-NOARG (0.01-10.0 μM) were preincubated with the appropriate homogenate (15 min on ice) prior to starting the reaction by addition of radiolabelled L-arginine (0.5 μCi, 120 nM). All four compounds caused dose-related inhibition of RC, BE and RL NOS enzyme activity. IC_{50} values (mean ± s.e. mean, n=6) in these experiments were as follows: 7-NI (RC, 0.71 ± 0.01 μM; BE, 0.78 ± 0.2 μM; RL, 5.8 ± 0.4 μM), 3-bromo 7-NI (RC, 0.17 ± 0.01 μM; BE, 0.86 ± 0.05 μM; RL, 0.29 ± 0.01 μM), 2,7 dinitro 7-NI (RC, 0.62 ± 0.01 μM; BE, 0.47 ± 0.04 μM; RL, 1.56 ± 0.06 μM) and L-NOARG (RC, 0.9 ± 0.1 μM; BE, 6.5 ± 1.1 μM; RL, 25.9 ± 0.06 μM). A number of additional substituted indazoles were also tested for ability to inhibit rat cerebellar NOS enzyme activity at a concentration of 10 μM. Inhibition of radiolabelled citrulline formation (all n=6) was as follows: 5,7-dinitro indazole (2H)-one (26.4 ± 1.6%), 1,6-dinitro indazole 3-carbonitrile (25.2 ± 1.7%), 3-ethyl indazole (18.8 ± 9.7%), 3-bromo 1,5-dinitro indazole (17.3 ± 2.2%), 6-nitro 1-phenyl indazole (15.4 ± 3.3%), 1-phenyl indazole (14.1 ± 4.7%), 7-amino indazole (7.2 ± 4.5%), 2,6-diethyl 6-nitro indazole 1-carboxanilide (5.2 ± 2.5%). Computerised molecular orbital calculation (software: HyperChem, AutoDesk Ltd., U.S.A.; computer: Silicon Graphics, model IRIS 4D 35/GT) to provide information concerning the atomic charge associated with individual atoms of indazole and related derivatives indicates a positive correlation between the relative (+) charge associated with the pyrazolo azo linkage (i.e. N1-N2) and the ability to inhibit rat cerebellar NOS.

P.B-W. is supported by a Glaxo Studentship.

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352P STRESS-RELATED CHANGES IN THE SHEEP INDUCED BY ICV INTERLEUKIN-1β: INCREASES IN PLASMA CORTISOL AND mRNA FOR CRH AND C-FOS IN THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS

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Although interleukin-1β (Il-1β) is known to stimulate stress hormone release via a central action (Rothwell, 1991), few experiments have been carried out in ungulate species and little is known about the molecular changes involved. Such studies are, however, important in relation to disease where high levels of Il-1β may be present in the circulation. Moreover, recently developed techniques which permit investigation of the expression of early-onset genes (e.g. c-fos; Vellucci & Parrott, in press) and stress hormone mRNA (Matthews *et al.*, 1991) in the ovine brain may provide important information on the underlying neural mechanisms.

Adult wether sheep (n=7) were surgically prepared under halothane anaesthesia with guide tubes aimed at the lateral cerebral ventricles to permit intracerebroventricular (icv) injection of human recombinant interleukin-1β (100ng, 1μg or 10μg) or saline vehicle. The animals were also provided with temporary catheters in the jugular vein for the collection of blood samples. Treatments were given in a random order to each animal and blood samples (10ml) were collected at 20 min intervals during the 60 min. before, and the 240 min. after, icv injection. Plasma obtained by centrifugation was stored at -30°C and analysed by radioimmunoassay to determine cortisol concentrations. The results indicated that only the highest dose of Il-1β had a significant effect ($p < 0.01$, ANOVA) on cortisol release. This increase was evident from 1 hour post-injection and was maintained throughout the rest of the sampling period with peak values which were 5-fold higher than in control animals.

In the second part of the experiment, sheep were given icv injections of Il-1β (10μg, n=5) or vehicle (n=2) and killed 120 min. later with intravenous barbiturate (Expiral). The brains were rapidly removed, frozen at -70°C and subsequently sectioned (12μm) on a cryostat. Sections were hybridised using a synthetic S^{35}-labelled oligonucleotide probe complementary to bases 1424-1468 of the ovine CRH gene (Matthews *et al.*, 1991), or complementary to homologous portions of the c-fos gene from the rat, mouse and man. Compared to controls, there was a clear upregulation of both c-fos and CRH mRNA levels in the paraventricular hypothalamic nucleus (PVN) and c-fos expression was additionally induced in the supraoptic and periventricular nuclei, lateral septal area, hippocampus and pyriform cortex.

These results confirm that centrally-administered Il-1β induces stress hormone responses in sheep. In addition, the effects of Il-1β on CRH and c-fos mRNA levels indicate that molecular approaches may be useful in future studies concerned with the identification of the neural substrates involved in stress and disease responses in this species.

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353P THE EFFECTS OF LIPOPOLYSACCHARIDE AND PROSTAGLANDINS ON IMMUNOREACTIVE INTERLEUKIN-1α RELEASE FROM RAT HYPOTHALAMIC EXPLANTS

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The pleiotropic cytokines interleukins-1 (IL-1) and -6 are produced during immune or inflammatory responses and neoplastic diseases. They act at the hypothalamic level to produce fever, anorexia and drowsiness, as well as activation of the hypothalamo-pituitary-adrenal axis, characteristic of the acute-phase response. The role of CNS-produced IL-1 in this response is, however, poorly understood. In previous short-term experiments, bioactive IL-1 was found to be released by rat hypothalamic explants. The IL-1-like bioactivity was mainly of the alpha type and its release could be modulated by bacterial lipopolysaccharide (LPS) and by prostaglandin E2 (PGE2) (Navarra et al., 1993). We have recently developed a specific radioimmunoassay for estimating IL-1α immunoreactivity (IL-1α-IR) released in vitro by rat hypothalamic explants. In the present study, these explants released measurable quantities (5-10 pg/hypothalamus/60-min) of IL-1α-IR which were significantly increased by 100 ng/ml LPS (but not by higher concentrations). In contrast, this release was dose-dependently inhibited by PGF2α, while PGE2 had no effect (Table 1).

TABLE 1 - The effects of LPS, PGE2 and PGF2α on IL-1α-IR release from rat hypothalamic explants over 60 minutes. Data are expressed as mean (± SEM; n=12) ratios of release in the presence of test substances to that observed in their absence. * P < 0.05 and ** P < 0.01 vs. Control. Student's t test.

Control	1.02 ± 0.09		Control	1.06 ± 0.11		Control	1.13 ± 0.11	
LPS 10 ng/ml	1.20 ± 0.08		PGE2 1 ng/ml	1.07 ± 0.14		PGF2α 1 ng/ml	1.10 ± 0.08	
100 ng/ml	1.34 ± 0.11	*	10 ng/ml	1.07 ± 0.16		10 ng/ml	0.86 ± 0.09	*
1 µg/ml	1.11 ± 0.15		100 ng/ml	0.86 ± 0.05		100 ng/ml	0.63 ± 0.07	**

Hypothalamic incubation appears to be a reliable model for investigating CNS synthesis and release of IL-1α-IR. This release can be specifically increased by LPS and inhibited by PGF2α. The latter however did not seem to exert a tonic inhibitory action on IL-1α-IR release, since blockade of cyclooxygenase by indomethacin had no effect on the cytokine's release under basal conditions (not shown).

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354P SUBTLE DIFFERENCES BETWEEN THE GLUTAMATE BINDING SITES OF CEREBELLAR *vs* FOREBRAIN NMDA RECEPTORS

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There is now both pharmacological and molecular biological evidence for multiple N-methyl-D-aspartate (NMDA)-type glutamate ion channels in mammalian brain. For example MK-801, TCP and PCP exhibit ten-fold lower affinities at cerebellar NMDA receptors as compared to forebrain regions (Vignon et al, 1986). Two NMDA subunits have been cloned in rodent brain which are selectively expressed in cerebellar granule cells (Seeburg, 1993). We therefore examined aspects of NMDA receptor pharmacology and regulation in cerebellum and forebrain to determine whether there are any differences between the two brain regions.

[^{3}H]L-Glutamate and [^{3}H]CGS19755 binding was performed in Tris-acetate buffer on extensively washed and Triton X-100-treated rat forebrain and cerebellar homogenates. Bound from free radioligand was separated by rapid centrifugation and the radioactivity estimated by liquid scintillation counting. Saturation analysis of NMDA-sensitive [^{3}H]glutamate binding in both forebrain and cerebellar homogenates showed binding to one site (Hill slopes close to unity) in each tissue, with similar densities. The affinity of [^{3}H]glutamate was, however, two fold significantly higher in the cerebellum, as compared to forebrain (cerebellum Kd = 110 ± 5.6nM; forebrain Kd = 216 ± 11nM * mean ± s.e.mean * P<0.001 Student's t-test). Competition of [^{3}H]glutamate binding with the agonists, L- and D-glutamate, L- and D-aspartate, NMDA and quisqualate showed between 2 and 7-fold higher affinities at cerebellar NMDA receptors as compared to forebrain NMDA receptors. Conversely, NMDA antagonists, such as CPP, CGS19755, stereoisomers of AP-5 and AP-7 exhibited the reverse relationship to the agonists, with a 2 to 5 fold lower affinity at cerebellar NMDA receptors as compared to forebrain NMDA receptors. Neither glycine agonists nor antagonists altered [^{3}H]glutamate binding in either forebrain or cerebellum. Glycine and D-serine inhibited [^{3}H]CGS19755 binding to the C5 antagonist recognition site of the NMDA receptor in both cerebellum and forebrain. The cerebellar NMDA receptors were much more sensitive to glycine and D-serine modulation as compared to forebrain NMDA receptors. In contrast, glycine antagonists, 7-chlorokynurate, 4,6-dichloroindole-2-carboxylate, 5,7-dichloro-2,4-dihydroxy-3-phenyl-quinoline and L689,560 all increased [^{3}H]CGS19755 binding in both cerebellum and forebrain. The ability of these glycine antagonists to increase [^{3}H]CGS19755 binding was greater in cerebellar homogenates as compared to forebrain. These results show that there are subtle differences in NMDA receptor pharmacology between cerebellum and forebrain. Moreover, modulation of [^{3}H]CGS19755 binding through the glycine site is enhanced in cerebellum vs. forebrain suggesting that the glycine binding site in the cerebellum exhibits different regulatory properties.

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355P PHARMACOLOGICAL CHARACTERIZATION OF THE BINDING OF THE GLYCINE ANTAGONIST [3H]5,7-DICHLOROKYNURENATE AND ITS MODULATION BY SPERMINE

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The N-methyl-D-aspartate (NMDA) receptor comprises various recognition domains for, among others, glutamate, its co-agonist glycine and polyamines. The glycine recognition domain has been probed using [^{3}H]glycine and, more recently, competitive glycine antagonists such as [^{3}H]5,7-dichlorokynurenate (DCKA; Baron *et al.*, 1991; Canton *et al.*, 1992). We have studied the pharmacological characteristics, including its modulation by the polyamine spermine, of [^{3}H]5,7-DCKA binding.

The binding of [^{3}H]5,7-DCKA (Canton *et al.*, 1992) was studied in the forebrain of male Sprague-Dawley rats (Iffa Credo, France). Well washed membranes (90 µg protein) were incubated with 10 nM of [^{3}H]5,7-DCKA (spec. act. 15.8 Ci/mmol; Dupont de Nemours/NEN, France) for 30 min at 4°C in 1 ml 50 mM HEPES-KOH (pH 8.0), recovered by filtration over Whatman GF/B glass fibre filters, and washed with three 4 ml aliquots of buffer. Nonspecific binding was determined in the presence of 1 mM glycine. [^{3}H]5,7-DCKA binding (K_d=25±4 nM; B_{max}=3.6±0.6 pmol/mg protein; n=3) is fully inhibited by (IC_{50}, µM) glycine (0.25) and D-alanine (3.2) and by the glycine antagonists 5,7-DCKA (0.039), 7-chlorokynurenate (0.18), DNQX (0.27), CNQX (1.4) and HA-966 (19). A significant correlation exists between the potency of these drugs to inhibit [^{3}H]5,7-DCKA and [^{3}H]glycine binding ($r = 0.90$; $p<0.01$). While spermine stimulates [^{3}H]glycine binding (Ransom, 1991), it partially inhibits [^{3}H]5,7-DCKA binding to 39±4 % of control values with an IC_{50} of 7.3±1.6 µM (n=4). The putative polyamine-site antagonists ifenprodil and eliprodil (SL82.0715) failed to affect [^{3}H]5,7-DCKA binding up to the highest concentration tested (100 µM). However, the inhibition of [^{3}H]5,7-DCKA binding seen in the presence of 10 µM spermine (48±6% of controls) is reversed by ifenprodil with an EC_{50} of 0.16±0.05 µM (n=4).

The present data support the use of [^{3}H]5,7-DCKA to label the strychnine-insensitive glycine recognition domain of the NMDA receptor and show that also the binding of radiolabelled glycine antagonists is subject to polyamine modulation. Similarly to the glutamate site of the NMDA receptor (Pullan & Powel, 1991), spermine reciprocally modulates the binding of glycine and glycine antagonists.

The authors ackowledge the expert technical assistance of Mrs. Michèle Bas.

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356P L-CPPene INHIBITION OF POTASSIUM-INDUCED RELEASE OF HIPPOCAMPAL GLUTAMATE *IN VIVO* IS REVERSED BY AN AP4 ANTAGONIST, RS-α-METHYL-4-CARBOXY-PHENYLGLYCINE

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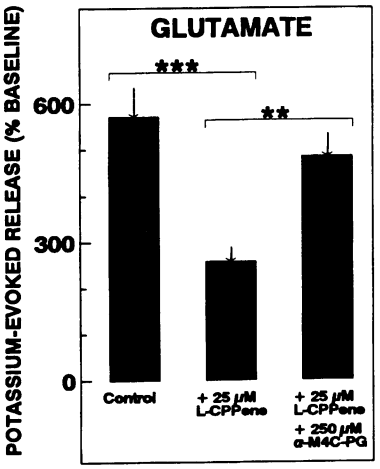


*Figure 1. The effect of 100 mM KCl on hippocampal interstitial glutamate levels (Control) in the presence of 25 µM L-CPPene +/- 250 µM α-M4C-PG. The values are expressed as % above baseline (** p<0.01; *** p<0.001)*

CPPene ((E)-4-(3-phosphono-2-propenyl)-piperazine-2-carboxylic acid) is a potent competitive antagonist of the NMDA receptor, with the D-enantiomer being 10 times more potent than the L-enantiomer in binding studies (Aebisher et al., 1989).

We have recently shown that, whereas the D-form of CPPene is the more potent anticonvulsant, L-CPPene is approximately 10 times more potent than the D-form in selectively inhibiting potassium-evoked release of hippocampal glutamate and aspartate in freely moving rats when 2.5, 25 and 250µM D- or L-CPPene were applied via dialysis probe together with a 100mM KCl (10 min) stimulus (Millan et al., 1994). The increases in extracellular aspartate and glutamate concentrations evoked by high concentrations of potassium were significantly reduced in the presence of 25µM and 250µM L-CPPene. D-CPPene produced significant inhibition of the release of glutamate and aspartate only at the highest concentration (250µM).

We here show that when 250µM RS-α-methyl-4-carboxy-phenylglycine (α-M4C-PG; an AP4 antagonist; Pook et al. 1993) was added to the perfusion medium during a 10 min pretreatment period followed by a 10 min high potassium stimulus in the presence of 25µM L-CPPene, there was a significant reversal of the CPPene effect on potassium-evoked increase in extracellular hippocampal glutamate level. A control 100mM KCl stimulus increased extracellular glutamate to 572% of baseline level. In the presence of 25µM L-CPPene the potassium-induced glutamate increase was reduced to 257% of baseline. The potassium-evoked increase in extracellular glutamate level was partially and significantly restored by 250µM α-M4C-PG to 487% of baseline level (Fig. 1). Reversal of L-CPPene inhibition by α-M4C-PG suggests that L-CPPene acts presynaptically at a metabotropic (AP4) receptor.

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357P ANTICONVULSANT EFFECTS OF FELBAMATE IN DBA/2 MICE: A POSSIBLE INVOLVEMENT OF NMDA AND NON-NMDA RECEPTOR COMPLEXES

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(Introduced by B.S. Meldrum)

Felbamate is a new anticonvulsant drug which has been found to reduce seizure frequency and severity in patients with uncontrolled complex partial seizures (Leppik *et al.*, 1989). This drug has been shown to possess an anticonvulsant activity against various models of experimental epilepsy (Swinyard *et al.*, 1986; White *et al*, 1992). The aim of present study was to evaluate the involvement of excitatory amino acids on the anticonvulsant activity of felbamate or vehicle to a sound of mixed frequency of 12-16 kHz, 109 dB intensity for up to 60 s. This auditory stimulation triggers a characteristic sequence of seizure response consisting of wild running, clonus, tonus and frequently respiratory arrest (De Sarro *et al*, 1984). In order to investigate the involvement of the glycine/NMDA receptor complex in the anticonvulsant action of felbamate, D-serine (300 nmol per mouse) was administered intracerebroventricularly (i.c.v.) concomitantly with various doses of felbamate. In addition, the anticonvulsant activity of felbamate was evaluated against seizures induced by i.c.v. administration of α-amino-3-hydroxy-5methyl-4-isoxazole propionic acid (AMPA), an agonist at non-NMDA receptors. Felbamate (25-300 mg/kg, i.p.) exerted anticonvulsant effects against sound induced seizures in DBA/2 mice. The ED50 values for suppression of tonic, clonic and wild running phases of the audiogenic seizures were 23.1, 48.8 and 114.6 mg/kg i.p respectively. Felbamate was able to prevent the seizures induced by i.c.v. administration of AMPA with ED50 values for suppression of tonic and clonic components of AMPA-induced seizures of 11.8 and 20.9 mg/kg i.p. respectively. Co-administration with D-serine, a glycine/NMDA receptor agonist, was able to increase by 1.9-3.4 times the ED50 values of felbamate. Specifically, the DBA/2 mice receiving felbamate + D-serine had as ED50 values for suppression of tonic and clonic components of the audiogenic seizures of 78.1 and 90.3 mg/kg i.p. respectively. The present data suggest that the interaction of felbamate with both NMDA and non-NMDA receptors may underlie the anticonvulsant properties of felbamate in the audiogenic DBA/2 mouse model.

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358P L-ARGININE AND GLYCINE MODULATE N-METHYL-D-ASPARTATE (NMDA)-INDUCED RELEASE OF GLUTAMATE FROM RAT STRIATAL SLICES

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Nitric oxide (NO) has been identified as having a physiological role in the CNS following NMDA receptor activation (Garthwaite *et al.*, 1989) and radioligand binding studies have shown NMDA receptors to be present in the striatum (Greenamyre *et al.*, 1985). It is therefore possible that NO may act as a regulatory molecule in this region and this study investigated the relationship between NMDA receptor activation, glutamate release and NO in rat striatal slices.

Coronal slices (250μm) were prepared from striata of female Wistar rats (200-250 g) using a McIlwain tissue chopper and then perfused with gassed (95% O_2/ 5% CO_2) artificial cerebrospinal fluid (aCSF) at 37°C. Two-minute aliquots of perfusate were collected and assayed for glutamate by HPLC with fluorometric detection. Transmitter release was elicited with a fourteen-minute pulse of NMDA (500μM) in magnesium-free aCSF and the results presented as the mean stimulated release per two-minute sample as a percentage of basal release.

NMDA on its own (n=9), or in the presence of glycine (100μM, n=13), or L-arginine (1mM, n=9) produced significant ($P<0.05$ - <0.01) increases of approximately 150%, 200% and 350% respectively in glutamate release. Consequently, glycine (100μM) was added to the magnesium-free aCSF in all subsequent experiments in order to potentiate NMDA receptor activity. In these experiments, NMDA in the presence of arginine (1mM) significantly decreased glutamate release (n=9, $P<0.01$) when compared to control (NMDA with glycine). Haemoglobin (1μM) reduced the NMDA-stimulated release of glutamate to basal levels in the presence of glycine, whereas 10μM significantly potentiated release (n=7, $P<0.05$) over both basal and NMDA-induced release.

NO is considered to be a possible retrograde messenger in the CNS following NMDA receptor activation and has been shown to release glutamate from cortical slices (Rowley *et al.*, 1993). However, recent evidence suggests that NO also blocks NMDA receptors in cortical neurones (Manzoni *et al.*, 1992). These present results suggest that at high levels of NO, following perfusion with 1mM arginine in the presence of glycine, glutamate release is possibly inhibited due to the blockade of the NMDA receptor by NO. The effect of Hb at 10μM in potentiating release may be due to its ability to bind NO and thereby reverse the blockade of the receptor. These results provide further evidence for NO being a modulator with the ability to regulate its neuronal environment by both increasing or decreasing transmitter release.

Acknowledgement: NJK is supported by a Wellcome Trust Studentship.

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359P KAINATE MODULATION OF [^{3}H]GLUTAMATE RELEASE FROM RAT HIPPOCAMPAL SYNAPTOSOMES

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The present study investigated the effect of kainate (KA) on the release of [^{3}H]glutamate from rat hippocampal synaptosomes to further investigate the ability of KA to modulate the release of glutamate (Solyakov et al., 1992).

The hippocampus was removed from female Wistar rats (150-200 g; Birmingham bred) and synaptosomes prepared according to the method of McMahon and colleagues (1992). The synaptosomes were incubated in gassed (O_2/CO_2; 95/5%) Krebs buffer (mM; NaCl 118.0; KCl 4.75; KH_2PO_4 1.2; $MgSO_4$ 1.2; $CaCl_2$ 2.5; $NaHCO_3$ 25.0, glucose 11.0) plus [^{3}H]glutamate (25 nM; 50 Ci/mmol) at 37°C for 7 min. The reaction was stopped by three washes in 10 volumes of ice-cold Krebs buffer. [^{3}H]Glutamate-loaded synaptosomes were immediately incubated in either Krebs buffer or high KCl-Krebs buffer (additional 20 mM KCl) in the absence (control) or presence of drugs for 2 min. The 'released' [^{3}H]glutamate was separated from the [^{3}H]glutamate associated with the synaptosomes by filtration under vacuum through GF/B filters followed by washing with an aliquot (1 ml) of ice-cold Krebs buffer. Radioactivity was assayed by liquid scintillation spectroscopy.

The majority of the KCl-stimulated [^{3}H]glutamate release (approx. 80 %) was calcium-dependent. KA (0.1-1000 μM) inhibited KCl-stimulated [^{3}H]glutamate release in a concentration-dependent manner (Table 1). The KA (100 μM)-induced inhibitory response was antagonised by the competitive non-NMDA receptor antagonist, CNQX (100 μM; 6-cyano-7-nitro-quinoxaline-2,3-dione; 92±8% control KCl-stimulated [^{3}H]glutamate release; mean±SEM, n=3) whereas CNQX alone failed to modify KCl-stimulated release (100 μM; 109% control KCl-stimulated [^{3}H]glutamate release; mean, n=2).

[KA] μM	% Control KCl-stimulated [^{3}H]glutamate release
1000	25±9
100	41±9
10	64±6
1.0	112±19
0.1	110±22

Table 1. Ability of KA to modulate KCl-stimulated [^{3}H]glutamate release from rat hippocampal synaptosomes. Drug-induced responses are compared to the KCl-stimulated [^{3}H]glutamate release in the absence of drug (control). Data represents the mean±SEM from 3-9 independent experiments.

This study is consistent with previous literature (e.g. Solyakov et al., 1992) which also demonstrated that KA inhibited [^{3}H]glutamate release in rat cerebral cortex synaptosomes and further implicates the presence of presynaptic KA receptors in the modulation of glutamate release.

This study was supported by The Wellcome Trust.

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360P EFFECTS OF EXCITATORY AMINO ACIDS ON GROWTH HORMONE SECRETION FROM RAT PITUITARY TISSUE *IN VITRO*

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Excitatory amino acids (EAAs) have been shown to stimulate growth hormone (GH) secretion in vivo (Mason et al. 1983) but it is unclear whether they exert their effects by increasing GH-releasing hormone (GHRH) release from the hypothalamus or via a direct action on the adenohypophysis to stimulate GH release. Thus in this study we have examined the effects of EAAs on basal and stimulated GH secretion from rat anterior pituitary segments in vitro.

The tissue was collected post-mortem from adult male Sprague-Dawley rats (≈200g) and, following a 2h pre-incubation period, incubated in the presence and absence of various test substances using a 60 min contact time (McAllister et al. 1993). GH released into the medium was determined by enzyme linked immunosorbent assay (ELISA) and is expressed (mean ± sem) as ng released/mg tissue. Hypothalamic extracts (0.05-0.4 HE/ml) and GHRH (1-100nM) caused significant ($p<0.005$) concentration dependent increases in GH release, with a submaximal concentration of GHRH (10nM) producing 2 to 3 fold increases in GH secretion. The EAAs, L-glutamate (10μM-1mM) and N-methyl-D-aspartate (NMDA, 10μM-1mM) generally failed to influence basal GH secretion, although in one instance resting GH release was inhibited by 100μM NMDA (from 198.4±26.0 to 133.2±14.0, $p<0.05$) and the lower concentrations of L-glutamate (control: 62.8±9.2 vs 10μM L-glutamate: 32.6±4.8, $p<0.05$; control: 62.8±9.2 vs 100μM L-glutamate: 41.9±2.8, $p<0.05$) whilst 1mM L-glutamate precipitated a significant ($p<0.001$) increase in GH release (from 15.8±1.9 to 49.6±4.8). The stimulatory responses to a submaximal concentration of hypothalamic extracts (0.1HE/ml) were unaffected by L-glutamate (10μM-1mM) but those to a submaximal concentration of GHRH (10nM) were potentiated ($p<0.05$) at least 1.5 fold by L-glutamate (1mM). The effects of L-glutamate on GHRH-induced GH secretion were mimicked by NMDA (100μM & 1mM) which augmented ($p<0.05$) the response to GHRH (10nM) approximately 1.7 fold. Omission of Ca^{2+} from the final incubation medium failed to influence basal or GHRH (10nM)-induced GH secretion but prevented the synergistic effects of L-glutamate (1mM) and GHRH (10nM) (control L-glutamate + GHRH: 143.7±28.2 vs Ca^{2+} free L-glutamate + GHRH: 67.1±11.3, $p<0.05$). Surprisingly, excess Mg^{2+} (3mM & 5mM) in the final incubation medium failed to reduce the stimulatory responses of GHRH (10nM) and L-glutamate (1mM) in combination (control: 130±54.8 vs 5mM Mg^{2+}: 57.6±17.6, N.S.). However Mg^{2+} (3mM) reduced basal (control:15.7±1.4 vs 3mM Mg^{2+}: 5.0±1.7, $p<0.05$) and GHRH (control: 34.9±6.6 vs 3mM Mg^{2+}: 10.33±1.0, $p<0.005$) induced GH secretion.
These results suggest that EAAs may act synergistically with GHRH to stimulate GH release from the rat anterior pituitary gland. Their effects may be mediated via NMDA receptors although the involvement of other EAA receptor subtypes can not be ruled out at this stage.

We are grateful to the Wellcome Trust for financial support and to NIDDK for the reagents for the GH ELISA.

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361P THE NMDA RECEPTOR ANTAGONISTS (±)-CPP AND CGS 19755 ATTENUATE THE BEHAVIOURAL AND NEUROCHEMICAL EFFECTS OF AMPHETAMINE IN MICE

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Previous studies have shown that the glycine/NMDA receptor antagonist, R-(+)-HA-966, selectively modulates mesolimbic dopaminergic systems in rodent brain (Hutson et al., 1991). Given that at least 3 distinct classes of NMDA receptor antagonist exist, we have now examined the effects of the competitive NMDA receptor antagonists, CGS 19755 and (±)-CPP on amphetamine induced hyperactivity and dopamine synthesis in the mouse.

Male BKTO mice (20 - 30 g) were habituated to individual photocell activity cages for at least 2 hours and then pretreated with either saline (10 ml/kg, i.p.), CGS 19755 (0.3 - 10 mg/kg, i.p.) or (±)-CPP (0.3 - 10 mg/kg, i.p.) 30 min prior to injection of either saline (10 ml/kg, s.c.) or d-amphetamine sulphate (5 mg/kg, s.c.). Locomotor activity was quantified by recording the number of crossings in 10 min intervals for 2 hours. DOPA accumulation was measured in mouse nucleus accumbens (n. acb.) and striatum following inhibition of aromatic amino acid decarboxylase with 3-hydroxybenzyl-hydrazine (NSD 1015). Animals were injected with either saline (10 ml/kg, i.p.), CGS 19755 (2 or 5 mg/kg, i.p.) or (±)-CPP (5 mg/kg, i.p.) followed 30 min later with either saline (10 ml/kg, s.c.) or amphetamine (2.5.mg/kg, s.c.). Fifteen min later, all mice received NSD 1015 (100 mg/kg, i.p.) and were killed after a further 25 min. DOPA content was measured by HPLC with electrochemical detection. Data were analysed using analysis of variance followed Tukey's t test.

Amphetamine injection significantly increased the number of cage crossings from 12 ± 1 counts/120 min in saline-treated controls to 806 ± 133 counts/120 min (mean ± s.e. mean of two experiments combined, n = 22 per group). Pretreatment with CGS 19755 or (±)-CPP dose-dependently attenuated amphetamine-induced locomotor activity, doses of 10 mg/kg reducing the response to 38 ± 9 and 332 ± 126 counts/120 min respectively (n = 9-12 per group). Neither compound impaired spontaneous locomotor activity at the doses used. Amphetamine injection also increased DOPA accumulation in mouse n. acb and striatum (saline/saline = 1.48 ± 0.07 µg/g, saline/amphetamine = 2.55 ± 0.15 µg/g for n. acb. and 1.05 ± 0.09 µg/g and 2.67 ± 0.15 µg/g for striatum respectively, n = 6-7 per group). CGS 19755 (2 mg/kg) significantly reduced this increase in DOPA accumulation in the n. acb. (1.75 ± 0.1 µg/g) but was without effect in the striatum (2.2 ± 0.2 µg/g). In contrast, (±)-CPP (5 mg/kg) significantly attenuated amphetamine-induced DOPA accumulation in both n. acb. (1.96 ± 0.09 µg/g) and striatum (0.99 ± 0.09 µg/g). Increasing the dose of CGS 19755 to 5 mg/kg also reduced this response in both brain regions.

These results provide further evidence that NMDA receptor antagonists attenuate amphetamine-induced activation of dopaminergic neurones. However, in contrast to (+)-HA-966, the effects seen with competitive antagonists may not be restricted to the mesolimbic dopamine system.

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362P RELATIVE AGONIST POTENCIES AT NEURONAL NICOTINIC RECEPTORS: A COMPARISON BETWEEN GANGLIONIC AND RECOMBINANT RECEPTORS

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Rat superior cervical ganglion (SCG) neurones express messenger RNAs for the $\alpha3$, $\alpha4$, $\alpha7$, $\beta4$ and $\beta2$ subunits of the nicotinic acetylcholine (ACh) receptor (Rust et al., 1994) but it is not clear which subunits make up the assembled receptor. We compared the potency of a range of nicotinic agonists on native SCG receptors and on receptors expressed in *Xenopus laevis* oocytes with $\alpha3\beta2$ or $\alpha3\beta4$ subunit pairs.

Whole-cell clamp recordings were obtained from undissociated SCG treated with trypsin (1 mg/ml, 15 min), followed by collagenase, trypsin inhibitor (both 1 mg/ml) and thermolysin (0.1 mg/ml, 45 min) and by cholinesterase inhibition with methanesulphonylfluoride (150 μM, 5 min). Oocyte responses were recorded using a two-electrode voltage clamp 3-6 days after nuclear injection of cDNAs (1:1 α:β ratio). Agonists were bath applied at -60 mV in the presence of 0.5 μM atropine (for oocytes, in mM: NaCl 115, $MgCl_2$ 1.8, KCl 2.5, HEPES 10; pH 7.2; for SCG: NaCl 125, KCl 2.5, $NaHCO_3$ 26, NaH_2PO_3 1.25, $MgCl_2$ 1, glucose 25, pH 7.4, bubbled with 95%:5% O_2:CO_2 and containing 100 nM tetrodotoxin). Potency ratios were estimated by fitting parallel curves to the low concentration end of the dose-response curves of two or more agonists.

Table 1 shows that the agonist pharmacology of SCG receptors is similar to that of the $\alpha3\beta4$ receptor (see especially the high potency of cytisine), except for the relatively high potency of 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) in the SCG.

Table 1. Potency ratio of nicotinic agonists relative to ACh

	cytisine	DMPP	nicotine	lobeline	carbachol
SCG neurones (n=3-4)	4.76 ± 0.70	2.50 ± 0.24	1.98 ± 0.13	0.066 ± 0.014	0.361 ± 0.022
Oocytes: $\alpha3\beta4$ (n=4-7)	5.06 ± 0.76	0.43 ± 0.08	1.10 ± 0.31	0.18 ± 0.04	0.25 ± 0.04
Oocytes: $\alpha3\beta2$ (n=7-21)	0.020 ± 0.012	1.62 ± 0.49	0.038 ± 0.012	0.207 ± 0.081	0.068 ± 0.028

Supported by the MRC. We are grateful to Dr J. Patrick for cDNAs and to The Sandoz Institute for housing the *Xenopus*.
Rust,G., Burgunder, J.M., Lauterburg, T.E. & Cachelin, A.B. (1994) *Eur. J. Neurosci.* in the press.

363P CHOLINERGIC STIMULATION OF ISOLATED EQUINE BRONCHI, THE EFFECT OF EPITHELIUM REMOVAL AND TISSUE WEIGHT

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Epithelial removal *in vitro* has been shown to potentiate the response of many contractile agonists in respiratory smooth muscle (Goldie *et al.* 1990) and it has been suggested that the diffusion barrier of the epithelial layer might contribute to this effect (Yang *et al.* 1991). However there is apparent variation in the ability of different cholinergic agonists to induce the potentiation (Morrison & Vanhoutte, 1992). In addition, bronchial size may affect the responsiveness to cholinergic stimulation, thus smaller bronchi may be more sensitive to an exogenous agonist (Stuart-Smith & Vanhoutte 1987).

To investigate these properties in the horse, bronchi obtained post-mortem were dissected to a uniform length of 3.5mm, suspended in modified (glucose 11.1mM/l) Kreb's solution (gassed with 5%CO_2/95%O_2). Isometric tension was recorded. On completion of each experiment, wet weight was measured. Epithelial removal was achieved via gentle rubbing with a moist splint of wood. Results are expressed as a proportion of the maximal contraction to 0.1mM agonist. Mann-Whitney analysis was performed on the EC_{50} values for dose response curves.

Epithelial removal potentiated the response of smaller bronchi to acetylcholine but not to carbachol or methacholine (Table 1).

Agonist	With epithelium 4-10mg	Without epithelium 4-10mg	With epithelium 10-20mg	Without epithelium 10-20mg
Ach	0.75uM+/-0.20,n=11*	0.33uM+/-0.20,n=5*	1.05uM+/-0.36,n=17	0.75uM+/-0.40,n=8
Meth	0.22uM+/-0.06,n=4	0.22uM+/-0.07,n=7	0.23uM+/-0.03,n=4	0.15uM+/-0.03,n=5
Carb	0.40uM+/-0..20,n=6	0.31uM+/-0.24,n=4	0.90uM+/-0.28,n=8	0.85uM+/-0..43,n=6

Table 1. Mean EC_{50} values (+/-S.E. mean). P<0.05 *

There was no significant effect of tissue weight on the response to cholinergic agonists, (Table 2).

Agonist	Wet weight 4-10mg	Wet Weight 10-20mg
Acetylcholine (Ach)	0.55uM+/-0.24,n=7	0.63uM+/-0.27,n=11
Methacholine (Meth)	0.22uM+/-0.06,n=4	0.23uM+/-0.06,n=4
Carbachol (Carb)	0.40uM+/-0.20,n=6	0.90uM+/-0.28,n=8

Table 2. Mean EC_{50} values (+/-S.E. mean).

The data presented here on the effect of epithelium removal is not conclusive, although the limited effect in equine bronchi may give support to the theory that the epithelium functions as a diffusion barrier. In addition there was no relationship between responsiveness of equine bronchi and tissue weight.

Acknowledgement: This work was funded by the Home of Rest for Horses.

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364P CHARACTERIZATION OF [^{3}H]Ba 679 BR, A SLOW-DISSOCIATING MUSCARINIC ANTAGONIST, IN HUMAN LUNG: RADIOLIGAND BINDING AND AUTORADIOGRAPHIC MAPPING

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Muscarinic receptors play an important role in human airway smooth muscle contraction and submucosal gland secretion. Patients with asthma and chronic obstructive airways disease suffer from bronchoconstriction and enhanced mucus production which can be relieved by cholinergic antagonists, such as ipratropium bromide. Ba 679 BR [7(S)-(1α,2ß,4ß,5α,7ß-7-[(hydroxydi - (2-thienyl) acetyl)-oxy] -9,9- dimethyl -3- oxa -9- azoniatricyclo [3.3.1.$0^{2,4}$] nonane bromide] is a new muscarinic antagonist developed as a bronchodilator drug with a long duration of action (Disse *et al.*, 1993). In this study, we have evaluated its affinity, selectivity and the distribution of its binding sites in human lung. [^{3}H]Ba 679 BR binds to a homogeneous population of muscarinic receptors in human lung membranes with affinities in the subnanomolar concentration range (K_d of 38.6 ± 9.7 pM and B_{max} of 48 ± 7 fmol/mg prot.). Like ipratropium bromide, Ba 679 BR shows no selectivity in its interaction with rat cerebrocortical M_1-receptors or heart M_2 and salivary gland M_3-receptors. Ba 679 BR displays 6 to 20 fold higher affinity compared to ipratropium bromide. We have also investigated the dissociation profile of Ba 679 BR from human lung muscarinic receptors as a potential mechanism whereby this drug achieves its duration of action. The dissociation of Ba 679 BR and ipratropium bromide from human lung muscarinic receptors was measured indirectly by monitoring the rate of [^{3}H]NMS association. Unlike ipratropium bromide (100 nM) which dissociates so quickly that there is little difference in the [^{3}H]NMS association to vehicle treated membranes, Ba 679 BR (1 nM) has a strong protective effect against [^{3}H]NMS binding (>70%) which lasts for 90 min. This result suggests that [^{3}H]NMS binding to human lung muscarinic receptors was probably limited by the slow dissociation rate of Ba 679 BR. Kinetic experiments conducted with [^{3}H]Ba 679 BR confirm the slow dissociation profile of this compound. The dissociation rate constant (k_{-1}) for [^{3}H]Ba 679 BR is (3.29 ± 0.18) x 10^{-3} min^{-1} corresponding to a half-life of the complex ($t_{½}$) of 212 ± 11 min. Autoradiographic studies reveal that [^{3}H]Ba 679 BR binding sites are densely distributed in alveolar walls and submucosal glands. These results suggest that the slow dissociation profile of Ba 679 BR from human lung muscarinic receptors might be the underlying mechanism by which this drug achieves its long duration of action observed in functional tests (Disse *et al.*, 1993).

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365P MODULATION BY MUSCARINIC RECEPTORS OF [^{3}H]ACETYLCHOLINE RELEASE FROM ISOLATED HUMAN BRONCHI: EFFECT OF INDOMETHACIN

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Release of acetylcholine from parasympathetic nerves innervating visceral organs is regulated by neuronal muscarinic autoreceptors. In the present experiments it was tested whether release of acetylcholine can be measured from human bronchi using a radiotracer method (Wessler et al., 1993) and whether acetylcholine release from human airways is regulated by muscarinic receptors at all. Moreover, the effect of blockade of cyclooxygenase activity on the action of muscarinic receptor agonists was investigated. Human tissue was obtained at thoracotomy from patients with lung cancer. In hospital, macroscopically normal bronchi (diameter of 3 - 6 mm) were dissected and placed in oxygenated salt solution within 5 to 20 min after surgical resection; airway mucosa was removed mechanically in most experiments. Bronchial tissue was set up in an organ bath, incubated with [^{3}H]-choline (30 min) and after a subsequent washout period tritium efflux was measured in 3 min periods. Release of [^{3}H]acetylcholine was elicited by four periods (S_1-S_4) of transmural electrical stimulation (four 20 s trains at 15 Hz during the individual stimulation periods). Increasing concentrations of oxotremorine were added 18 min before S_2, S_3, S_4; in the respective experiments the muscarinic receptor antagonist pirenzepine and the cyclooyxgenase inhibitor indomethacin were added 30 min before S_1.

Transmural electrical stimulation caused an increase in tritium efflux that was abolished by removal of extracellular calcium or by addition of tetrodotoxin (1 μM.) Reverse phase HPLC was used to separate the different radioactive compounds of the bath efflux obtained in the presence of 1 μM neostigmine; the stimulated increase in total tritium efflux could be attributed to an enhanced efflux of [^{3}H]acetylcholine (2900 ± 750 dpm /100mg total tritium vs. 2300 ± 630 dpm/100 mg [^{3}H]acetylcholine; n = 5), whereas the efflux of [^{3}H]choline or [^{3}H]phosphorylcholine was not affected by transmural stimulation. Oxotremorine produced two concentration-related effects: the agonist increased the basal tritium efflux immediately after its application and inhibited the release of [^{3}H]acetylcholine evoked by a subsequent electrical stimulation (IC_{50} value of 100 nM). Indomethacin (3 μM) affected both effects of oxotremorine differently: the facilitatory effect was significantly enhanced, whereas the inhibitory effect of a low oxotremorine concentration (10 nM) was abolished. Indomethacin, however, did not modify the inhibitory effect of high oxotremorine concentrations (0.1, 1 μM). In the presence of indomethacin pirenzepine shifted the concentration-response curve for the facilitatory effect to the right in a manner suggesting competitive interaction; the slope of the Schild plot did not differ from unity and a pA_2 value of 8.1 was obtained. Pirenzepine antagonized also the inhibitory effect of oxotremorine but a sluggish Schild plot was obtained. Pirenzepine, alone (added 18 min before S_2), did not significantly modify evoked [^{3}H]acetylcholine release under the present experimental conditions.

Release of newly-synthesized acetylcholine can be measured from human bronchi. Both inhibitory and facilitatory muscarinic receptors are present in human airways, facilitatory receptors of the M_1-subtype enhance basal tritium efflux reflecting most likely an enhanced basal release of acetylcholine. Evoked acetylcholine release appears to be controlled in a complex manner by different muscarinic receptors. The interaction experiments with oxotremorine and indomethacin suggest that applied muscarinic receptor agonists inhibit evoked acetylcholine release by two mechanisms, directly via presynaptic receptors and indirectly via the simultaneous generation of inhibitory prostanoids which inhibit also the M_1-evoked basal tritium efflux.

Supported by the Deutsche Forschungsgemeinschaft.

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366P SPONTANEOUSLY HYPERTENSIVE RATS WITH DIABETES: ENDOTHELIUM-DEPENDENT AND -INDEPENDENT RELAXATION OF ISOLATED SMALL ARTERIES

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Diabetes mellitus and hypertension are known to coincide frequently. Growing awareness of the importance of the integrity of the endothelium for the maintenance of cardiovascular homeostasis has led to studies concerned with the possibility that endothelium-dependent vasorelaxation may be abnormal in diabetes and hypertension. We investigated endothelium-dependent (EDR) and -independent relaxation (EIR) in isolated small arteries of spontaneously hypertensive rats (SHR) with simultaneous chronic (8 weeks) diabetes. SHR and normotensive Wistar-Kyoto rats (WKY) with streptozotocin-induced diabetes (60 $mg \cdot kg^{-1}$ i.v.) were used. Mean arterial pressures (MAP, carotid artery) of both control (means ± SEM, $n \geq 45$, 177.0 ± 4.7 mmHg) and diabetic (147.5 ± 5.4 mmHg) SHR were significantly ($p < 0.05$) higher compared to both control (100.4 ± 2.2 mmHg) and diabetic (110.4 ± 2.4 mmHg) WKY. Mesenteric small arteries were prepared and normalized to their individual optimal lumen diameter (±240 μm for all groups) in an isometric wire-myograph. The passive wall tension ($mN \cdot mm^{-1}$) of preparations taken from diabetic SHR ($n \geq 30$, 0.53 ± 0.05) and control WKY (0.41 ± 0.03) were significantly lower compared to control SHR (0.76 ± 0.05). Cumulative concentration-response curves (CRC) were constructed for sodium nitroprusside (SNP), methacholine and histamine, after precontraction with methoxamine ($10\ \mu mol \cdot l^{-1}$).

Table 1. pD_2-values and E_{max} (maximal relaxation, %) of CRC. $N \geq 6$, means ± SEM, *: $p < 0.05$ vs control or †: vs WKY

	control WKY		diabetic WKY		control SHR		diabetic SHR	
	pD_2	E_{max}(%)	pD_2	E_{max}(%)	pD_2	E_{max}(%)	pD_2	E_{max}(%)
SNP	6.86±0.07	94.3±1.4	7.01±0.06	93.2±3.2	6.85±0.06	95.7±0.8	6.90±0.09	94.1±0.7
methacholine	7.26±0.04	99.6±0.3	6.70±0.13	96.6±1.4	7.19±0.08	95.6±1.3	6.69±0.07	95.8±1.2
histamine	6.46±0.07	100.3±0.	6.94±0.16	99.2±0.6	6.04±0.15	97.2±1.0	6.48±0.25	95.4±2.4

In conclusion: the MAP of diabetic and non-diabetic hypertensive rats is significantly higher compared to appropriate normotensive control rats, whereas the induced diabetes elevated the MAP in the normotensive group and lowered it in the hypertensive group. Furthermore, the EIR in isolated mesenteric small arteries appears not to be influenced by hypertension or diabetes. The EDR caused by methacholine proved to be impaired (*, -logEC50-value) but the degree of E_{max} was fully preserved by the induction of diabetes. Hypertension however reduced the E_{max} in the vessels from the non-diabetic group. Since changes in the CRC for histamine showed a different pattern, we conclude that neither streptozotocin-induced diabetes mellitus nor hypertension *per se* will lead to an overall endothelial dysfunction, but are rather involved in disturbances of particular receptor systems.

367P STREPTOZOTOCIN-INDUCED DIABETES MELLITUS IN SPONTANEOUSLY HYPERTENSIVE RATS: A PATHOPHYSIOLOGICAL MODEL FOR THE COMBINED EFFECTS OF HYPERTENSION AND DIABETES

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The present study was undertaken to investigate the combined effects of hypertension and diabetes mellitus induced by streptozotocin (60 mg/kg intravenously) in normotensive Wistar Kyoto rats and spontaneously hypertensive rats, with the aim to develop an animal model reflecting the simultaneous occurrence of hypertension and diabetes. Accordingly, 4 groups of rats were studied: Wistar Kyoto rats (W), diabetic Wistar Kyoto rats (DW), spontaneously hypertensive rats (S) and diabetic spontaneously hypertensive rats (DS). The blood glucose level (mmol/l) was significantly ($p<0.05$) elevated in diabetic rats (W:6.9±0.3, DW:25.7±0.4, S:7.6±0.2, DS:24.4±0.5). The mean arterial pressure (mmHg) and heart rate (bpm) were significantly ($p<0.05$) increased in hypertensive rats (W:101.6±2.5/316.2±6.8, DW:107.6±4.4-/339.2±9.1, S:171.4±4.0/372.9±5.8, DS:160.0±4.7/371.9±8.0). Mortality rates (%) 8 weeks after treatment, just prior to sacrifice of the rats were : W:7.8, DW:5.7, S:3.1, DS 5.7. The base excess (mmol/l) was elevated in the diabetic animals (W:1.9±0.3, DW:3.5±0.9, S:0.5±1.0, DS:4.7±0.6) whereas the pH did not differ significantly between the groups and was within the normal range (W:7.43±0.02, DW:7.41±0.01, S:7.45±0.02, DS:7.46±0.01). This was measured in the arterial blood of conscious rats. Elevated concentrations of glucose and ketone bodies were found in the blood and urine of diabetic rats, when measured semiquantitatively.

In separate experiments isolated working hearts of the 4 groups were analyzed and concentration response curves were constructed for the influence on contractile force. For the maximal left ventricular pressure (mmHg) the following values were established: W:110.0±2.6, DW:93.6±2.7, S:93.4±3.0, DS:87.5±2.4). There was no difference in sensitivity among the various groups for nifedipine as reflected by the -log EC_{50} values for negative inotropy (W:6.84±0.03, DW:6.98±0.22, S:6.83±0.02, DS:6.87±0.01). The wet heart-, dry heart- and body weights of the diabetic rats were significantly lower compared to those of normoglycemic rats. The wet heart weight/body weight ratio was significantly increased by diabetes and hypertension (W:0.43±0.01, DW:0.47±0.01, S:0.47±0.01, DS:0.54±0.02). There was no difference in water content of the hearts from the 4 groups, consequently the weight loss was due to loss of cardiac tissue and not due to loss of water.

In histological sections of the hearts the media (calculated as ratio: external- minus internal diameter/2 x external diameter) of the intramyocardial vessels in diabetic and hypertensive rats was significantly thickened (W:0.09±0.00, DW:0.10±0.00, S:0.14±0.02, DS:0.15±0.02). The left ventricular wall (mm) was significantly thinner in hearts of diabetic rats (W:3.09±0.14, DW:2.24±0.09, S:3.40±0.07, DS:2.59±0.13). The left ventricular wall to lumen ratio was increased in diabetes (W:6.91±0.62, DW:4.40±0.82, S:8.46±1.44, DS:4.54±0.58). Both cardiac hypertrophy and -dilatation were observed in histological preparations. There was no difference in collagen content of the hearts between the various groups, as measured spectrophotometrically.

In conclusion: The animal model described offers a reasonable reflection of the human situation, where diabetes and hypertension often coincide. It is easy to handle, reproducible and suitable for pharmacological and biochemical investigations. One of its drawbacks is the fact that streptozotocin-induced diabetes rather resembles type 1 than type 2 diabetes, whereas in the human situation the simultaneous occurrence of both diseases more frequently concerns type 2 diabetes. The main advantage is the possibility to separately study the influence of diabetes and hypertension.

368P THE EFFECT OF *PHYLLANTHUS SELLOWIANUS* EXTRACT IN STREPTOZOTOCIN-DIABETIC RATS

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In South America the green parts of the sarandi blanco plant (Phyllanthus sellowianus Mull. Arg.) are brewed as a tea to treat the symptoms of diabetes mellitus (DM) (Bailey & Day, 1989). In 1954, Barbara Woodhouse broadcast her claim to successful treatment of insulin dependent diabetes by drinking the brew, and this has resulted in continued public interest in the possible curative property of the plant.

In this study, the potential antidiabetic activity of the plant extract was tested in adult male Long Evans rats with DM induced by streptozotocin (STZ, 60mg.kg^{-1} i.p.). Briefly, the plant extract (PSX) consisted of the filtrate obtained by adding 8.25g of freeze-dried leaves and shoots to 1.0 litre hot water. Once diabetes was established (blood glucose concentration > 15mmol.l^{-1}) rats were randomly divided into 2 groups (both n=4) and the drinking water of one group was replaced with PSX, the other group being maintained on tap water (controls).

Daily fluid and food intakes and body weights for both groups were not significantly different before PSX administration, but seven days after PSX the treated group had a significantly higher food intake (mean ± SEM, treated v. control: 45.5 ± 2.7 and 36.3 ± 0.7g respectively; $P<0.05$, Student unpaired t test) although fluid intakes and body weights were not significantly different. On days 11 and 13 the mean fluid intake was significantly greater ($P<0.02$) in the PSX treated rats than in the controls (day 11: 197.3 ± 8.3 v. 164.2 ± 5.4ml respectively; day 13: 203.2 ± 4 v. 171.4 ± 7.5ml respectively). Food intake was also significantly raised in the PSX-treated group ($P<0.01$) on day 11 (51.6 ± 2.2 v. 42.9 ± 2g), although body weights were not significantly different at all times. By day 15, however, all measured variables did not differ significantly between the two groups. There were no significant differences between the group mean blood glucose concentrations (days 3, 10 and 15: treated rats, 26.1 ± 1.54, 23.7 ± 1.3 and 20.2 ± 1.2 mmol.l^{-1}; controls, 26.9 ± 1.0, 22.8 ± 1.0 and 22.3 ± 1.7 mmol.l^{-1}).

These results indicate that PSX has no beneficial effect in reducing the blood glucose concentration, nor in reducing the polydipsia and polyphagia associated with DM in the type I animal model, the STZ-treated rat.

Supported by the British Diabetic Association.

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369P EFFECTS OF ISOENZYME-SPECIFIC PHOSPHODIESTERASE INHIBITORS ON INSULIN SECRETION IN THE RAT

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Inhibitors of Type III, but not of Type IV or type V, phosphodiesterase (PDE) isoenzyme stimulate glucose-induced insulin release from rat isolated islets (Shafiee-Nick et al. 1993). The aim of the present work was to compare the effects of a specific type III PDE inhibitor (Org 9935, de Boer et al., 1992) with those of a mixed type III/IV inhibitor (Org 30029, de Boer et al. 1993) on glucose-induced insulin secretion in vitro, plasma insulin concentrations and rat islet PDE activity.

Rat collagenase-isolated islets were incubated (batches of 5) with Krebs-bicarbonate buffer (37°C, pH 7.3-7.4, 95% O_2/5% CO_2, 1h) containing glucose (3 or 10 mM) with or without drugs or appropriate concentrations of vehicle (DMSO). Immunoreactive insulin (IRI) was assayed in the medium after 1h. Glucose (10mM) increased insulin release (insulin release, ng islet^{-1} h^{-1}; glucose 3mM 1.8 ± 0.2, glucose 10mM, 15.9 ± 1.2; $p < 0.01$). Org 9935 (0.1 - 100 μM) enhanced glucose-induced insulin release in a concentration-dependent manner, with an EC_{50} of about 1.5 μM (e.g.insulin release, ng islet^{-1} h^{-1}; control 15.3 ± 1.5, Org 9935 35.1 ± 5; n= 9, $p<0.01$). Org 30029 also increased glucose-induced insulin secretion but was less potent, with an EC_{50} of about 20 μM.

In 18h-fasted, pentobarbitone (60 mg kg^{-1} i.p.) anaesthetised rats, with cannulae in the carotid artery (for blood sampling) and femoral vein (for injection), Org 9935 (0.1 and 1 mg kg^{-1}, 15 min prior to glucose 0.25 g kg^{-1}, i.v.) increased both fasting plasma insulin (ng ml^{-1}, geometric mean, (95% CL); control 1.5 (1.2,2.0); Org 9935 (0.1 mg kg^{-1}) 7.6 (4.9, 11.8); $p < 0.01$) and the plasma insulin concentrations after glucose injection (e.g. at 10 min after glucose, control, 3.9 (2.3, 6.6), Org 9935 11.4 (6.9, 18.8); $p<0.05$). Org 30029 produced similar effects but only at a dose of 10mg kg^{-1}. Neither drug modified fasting or post-glucose plasma glucose concentrations.

Homogenates of rat islets were centrifuged (48600 g) and fractions assayed for cAMP PDE activity (Thompson & Appleman, 1971). Org 9935 potently inhibited PDE activity by a maximum of 81.2 ± 4.4% (control 47.1 ± 1.5 pmol min^{-1} ml^{-1}) in the pellet with an IC_{50} of 20 nM and by 54.3 ± 1.2% in the supernate (control 45.6 ± 1.7 pmol min^{-1} ml^{-1}) with an IC_{50} of 55 nM. Org 30029 was less potent with an IC_{50} of 50 - 100 μM in both pellet and supernate.

The increased insulin release in vivo and in vitro in response to Org 9935 and Org 30029 may be explained by inhibition of cAMP PDE, thus increasing the islet B-cell content of cAMP. The failure to modify blood glucose despite markedly raised insulin concentrations may be due to concomitant inhibition of PDE in liver and adipose tissue.

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370P PHARMACOLOGICAL MODULATION OF THE TRYPSIN MODIFIED K_{ATP} IN THE CRI-G1 INSULIN-SECRETING CELL LINE

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The enzyme trypsin has previously been shown to reverse the process of K_{ATP} run-down and to reduce the channels sensitivity to the sulphonylureas when applied to the intracellular surface of membrane patches excised from insulin-secreting cells (Proks & Ashcroft, 1993). In this study we have used both single channel and whole-cell recording techniques in order to examine the ability of various K_{ATP} inhibitors to modulate channel activity both prior to and after exposure of the channel to intracellular trypsin.

The application of 100μg/ml trypsin to the intracellular surface of inside-out patches excised from CRI-G1 cells induced an increase in channel activity to 345.7 ± 34.3% (n=15) of the control activity. Prior to trypsin application, the sulphonylureas tolbutamide (100μM) and glibenclamide (5μM) produced 95.2 ± 3.2 % (n=14) and 97.3 ± 5.7 % (n=4) inhibition of K_{ATP} activity respectively. However, following trypsinization, these agents were significantly less effective (8.4 ± 4.3 % (n=5) and 20.2 ± 11.3 % (n=4) inhibition, respectively; $P<0.05$). The barbiturate thiopentone (100μM) retained its inhibitory effectiveness even after exposure of the channels to trypsin (92.2 ± 2.6 % (n=8) inhibition before and 90.3 ± 3.4 % (n=4) inhibition after trypsin treatment).

Using the whole-cell configuration, it was possible to obtain concentration response curves for the above compounds in the presence and absence of trypsin in the electrode solution. In the absence of trypsin, tolbutamide inhibited whole-cell $I_{K(ATP)}$ with an EC_{50} of 12.1 ± 2.2μM. Similar values were obtained when 100μg/ml heat inactivated trypsin was introduced into the electrode solution or when 1mg/ml trypsin was applied to the cells external surface (30.0 ± 4.5μM and 47.7 ± 12.2μM, respectively). However, when 100μg/ml trypsin was introduced into the electrode solution, the EC_{50} was reduced significantly to 9.7 ± 1.0mM. A similar reduction in the inhibitory potency of glibenclamide was observed when trypsin was introduced into the electrode solution (2.1 ± 0.4nM to 1.0 ± 0.2mM). However this procedure did not affect the inhibitory effectiveness of thiopentone (36.8 ± 10.8μM and 69.5 ± 18.6μM) in the presence and absence of trypsin in the electrode solution respectively.

These observations lead us to suggest that the internal application of trypsin produces a change in the properties of the K_{ATP} such that its sulphonylurea sensitivity is altered by this treatment.

This work was supported by the Wellcome Trust, K.L. is a Wellcome prize student.

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371P PIOGLITAZONE, AN ORALLY ACTIVE ANTI-DIABETIC AGENT, DECREASES THE VASCULAR REACTIVITY OF OBESE ZUCKER RATS BUT NOT THAT OF SPONTANEOUSLY HYPERTENSIVE RATS

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An increased vascular reactivity is a common feature of animal models of hypertension. This has also been demonstrated in the obese Zucker rat, a model of insulin resistance (IR). Zemel et al. (1991) and Cox & Kikta (1992) both demonstrated an increased reactivity in-vitro of tissues from obese Zucker rats compared to tissues from their lean littermates. Pioglitazone, a thiazolidinedione antidiabetic agent, has been shown to increase insulin sensitivity in insulin resistant states (Ikeda et al., 1990). In order to determine whether this increased reactivity was related to the insulin resistant state of the animal it was decided to test the ability of pioglitazone to alter the vascular reactivity of three rat strains, the Alderley Park (AP) wistar (a normotensive strain), the AP spontaneously hypertensive (SH) rat and the obese Zucker rat.

Male rats, approximately six months of age, of the above strains were orally dosed, once daily, with either 30mg/kg pioglitazone or vehicle (polysorbate) for seven days prior to assessment of vascular reactivity. Rats were anaesthetised with Saffan anaesthetic (Pittman-Moore) at 6mg/kg via the tail vein. The rats were pithed via the eye-orbit and immediately ventilated with a 40% oxygen and 60% nitrogen mixture at a volume of 4ml and a frequency of 55 strokes per minute. The carotid artery was cannulated to allow blood pressure and heart rate measurements to be made. The jugular vein was cannulated to allow drug administration. Temperature of the preparation was maintained using a heated blanket and a rectal thermometer. Pressor response curves were generated using doses of 1, 3, 10, 30 and 100ug/kg i.v. phenylephrine.

Reactivity to phenylephrine in the obese Zucker rat was found to be approximately equal to that of the AP wistar but both these strains were less responsive than the SH strain tested e.g. at 30ug/kg; 88 ± 8.7, 96 ± 11.3, 125 ± 7.4 mmHg respectively. A significant decrease in pressor dose response was seen however in the pioglitazone treated obese Zucker rats (n=10) compared to their vehicle treated counterpart (n=9) e.g. at 10ug/kg; 57 ± 4.8 mmHg vs. 30 ± 5.7 mmHg ($P< 0.01$). No significant differences were detected between the pressor curves of the AP wistars (n=4) treated with pioglitazone and those treated with vehicle alone (n=5) nor between the pioglitazone treated SH rats (n=10) and the vehicle treated SH rats (n=9).

The results demonstrate that different mechanisms underpin the enhanced vascular reactivity of the obese Zucker rat and the SH rat. Unlike the SH rat, the obese Zucker rat was sensitive to the insulin sensitising agent pioglitazone. This suggests that blood pressure in the obese Zucker rat may be determined by the IR state of the animals and may, at least in part, be mediated by enhanced vascular reactivity involving peripheral mechanisms. Moreover it would also suggest that no such IR state determines reactivity in the SH rat strain tested.

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372P THE EFFECTS OF TREATMENT WITH ESSENTIAL FATTY ACIDS AND ANTI-OXIDANTS ON SCIATIC NERVE DYSFUNCTION IN STREPTOZOTOCIN-DIABETIC RATS

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This study compared the effects of evening primrose oil (EPO) or anti-oxidants on sciatic nerve dysfunction in experimental diabetes. Study 1 investigated the effects of treatment of diabetic rats with the anti-oxidants probucol or Vitamin-E (Vit-E; both 1% w:w by diet) on sciatic nerve laser Doppler flux (NLDF) as an index of nerve blood flow. Study 2 determined effects of EPO, either alone (0.3-0.4 ml/rat/day by gavage) or with probucol (1% w:w by diet), on NLDF and motor nerve conduction velocity (NCV). Rats were either made diabetic with STZ (50 mg/kg i.p) or left as controls. One diabetic group of each study remained untreated. Four to five weeks later, rats were anaesthetised with alphaxalone/alphadolone for measurement of sciatic NLDF and/or NCV, with concomitant recording of arterial pressure. Treatments did not influence the severity of diabetes as judged by weight loss and hyperglycemia. Untreated diabetes increased plasma cholesterol and triglyceride concentrations. Neither probucol nor Vit-E treatment of diabetes affected plasma cholesterol concentrations, but both attenuated raised plasma triglycerides. EPO treatment, alone or with probucol, attenuated the increases in plasma cholesterol and triglycerides of diabetes. Sciatic NLDF and NCV were significantly decreased in STZ-diabetic rats compared to control rats. This deficit was lessened by Vit-E and, to a greater extent, by probucol. EPO treatment, either alone or with probucol, increased sciatic NLDF and NCV of STZ-diabetes. Amelioration of the NLDF deficit was more successful with combined treatment than with EPO alone. All rats were normotensive.

Table 1.	Study 1				Study 2			
Groups	Control	Db	Db+P	Db+Vit-E	Control	Db	Db+EPO	Db+EPO+P
Cholesterol (mg/dl)	60 ± 12^{x}	91 ± 23^{y}	97 ± 21^{y}	90 ± 22^{y}	53 ± 7^{xa}	84 ± 12^{y}	68 ± 15^{xb}	65 ± 17^{xb}
Triglyceride (mg/dl)	127 ± 50^{xa}	313 ± 159^{y}	111 ± 39^{xa}	237 ± 108^{b}	70 ± 31^{a}	138 ± 63^{b}	96 ± 68	84 ± 33^{a}
NLDF (units)	389 ± 40^{xXa}	214 ± 34^{y}	338 ± 28^{xb}	328 ± 38^{xY}	395 ± 43^{xa}	208 ± 48^{y}	338 ± 63^{xb}	361 ± 67^{x}
NCV (m/s)	ND	ND	ND	ND	51 ± 8^{x}	36 ± 4^{y}	47 ± 6^{x}	47 ± 6^{x}

Data (mean ± SD) were analysed by ANOVA and differences within each study tested using Duncan's multiple range test where $p<0.05$; a:b and $p<0.01$; x: y, X:Y. ND = not determined, Db = diabetic, P = probucol and EPO = evening primrose oil.

In summary, either anti-oxidants or EPO can attenuate diabetes-induced deficits in sciatic nerve function. However, a full additive effect is not seen with EPO and probucol combined, suggesting that EPO may, in part, act as an anti-oxidant.

This study was supported by the William Harvey Research Institue via a donation from Ono Pharmaceuticals.

373P EFFECTS OF AMINOGUANIDINE TREATMENT ON SCIATIC NERVE LASER DOPPLER FLUX AND PROSTACYCLIN RELEASE FROM NERVE AND VASCULAR TISSUES OF CONTROL AND STREPTOZOTOCIN-DIABETIC RATS

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Streptozotocin (STZ)-diabetes in rats causes a deficit in sciatic nerve laser Doppler flux (LDF) which may be due to the defective release of vasoactive mediators such as prostacyclin (Stevens *et al.*, 1993). Preliminary studies have shown that aminoguanidine (AG) can increase reactive prostacyclin release from perfused rat lungs. The aim of this study was, thus, to investigate the effects of AG on LDF and prostacyclin release by sciatic nerve, aortic rings and femoral arteries. Male Wistar rats, (282 ± 9 g; all data mean ± 1 S.D.) were divided into three groups, an age-matched control, a diabetic (STZ 65 mg/kg i.p.) and an AG treated (1 g l^{-1} in drinking water) diabetic group and used 8 weeks later. Anaesthesia was induced using halothane and maintained by a bolus (4 mg kg^{-1}), followed by continuous infusion (12 mg h^{-1}), of alphaxolone:alphadolone (3:1). LDF was measured using a laser-Doppler probe at the nerve surface, with simultaneous recording of carotid arterial pressure. After measurement of LDF animals were perfused with heparinised (100 U ml^{-1}) Krebs'-Henseleit buffered saline (KHBS; pH 7.4, 37°C, gassed with 95 % O_2/5 % CO_2) and tissues removed, placed in unheparinised KHBS, and allowed to equilibriate for 30 min. Tissues were then incubated for 10 min in fresh KHBS followed by incubation in either KHBS alone (basal) or with arachidonic acid ($1x10^{-4}$ M; stimulated) for a further 10 mins. Aliquots of incubation media were assayed for immunoreactive 6-keto prostaglandin $F_{1\alpha}$, a stable metabolite of prostacyclin. LDF was significantly ($p<0.01$: arbitrary units) decreased in diabetic (219 ± 40; n=7) and AG treated (166 ± 50; n=8) diabetic rats compared to controls (383 ± 52; n=9) with no significant differences in arterial pressure (116 ± 11 for control, 115 ± 9 for diabetic and 122 ± 9 for AG-treated).

Table 1. Prostacyclin release by tissues *in vitro*. Data, (mean ±1 S.D; n=7 to 9 for each group), was analysed by analysis of variance and significance tested using Duncan's multiple range tests at $p<0.01$ (a v b).

	Basal release (ng min^{-1} mg $tissue^{-1}$)			Stimulated release (ng min^{-1} mg $tissue^{-1}$)		
Group	Sciatic	Femoral	Aortic	Sciatic	Femoral	Aortic
Control	0.11 ± 0.04	2.07 ± 0.99[a]	1.55 ± 0.86	1.12 ± 0.69	4.65 ± 0.54	19.15 ± 9.14
Diabetic	0.08 ± 0.04	0.86 ± 0.53[b]	1.74 ± 0.74	1.13 ± 0.91	5.74 ± 2.49	9.32 ± 5.23
AG-treated	0.08 ± 0.04	4.62 ± 0.79[b]	2.80 ± 1.72	1.09 ± 0.78	6.00 ± 2.51	29.89 ± 24.00

In summary, AG had no significant effect on LDF in the STZ-diabetic rat and concurrently did not affect mean arterial pressure or sciatic nerve prostacyclin production *in vitro*. AG did, however, enhance prostacyclin release by vascular tissue *in vitro*.

Stevens, E.J., Lockett, M.J., Carrington, A.L. & Tomlinson, D.R. (1993). Diabetologia. 36, 397-401.

This was supported by a grant from the William Harvey Research Institute made possible by a donation from Ono Pharmaceuticals.

374P THERMAL SENSITIVITY IN STREPTOZOTOCIN-DIABETIC RATS

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Sensory neuropathy is a common complication of diabetes in man. It is often associated with autonomic neuropathy and, like the latter, progresses with the duration of diabetes (Watkins, 1992). Several therapeutic approaches to treatment/prevention have been proposed e.g. aldose reductase inhibitors, gangliosides, gamma linoleic acid and myo-inositol. There are, however, serious difficulties attached to the assessment of their efficacies in diabetic patients. These arise, in part, from the unpredictable and, usually, slow development of diabetic neuropathy. None of the above therapies has yet been shown to be of clinical value. An appropriate animal model would clearly be useful.

In diabetic sensory neuropathy the extremities are generally affected first with loss of thermal sensitivity being an early symptom (Jensen et al., 1991). In short term (e.g. 8 weeks) diabetic rodents thermal sensitivity has been reported to be unchanged (Kamei et al., 1990) or increased (Forman et al., 1986), however, there appear to be no reports of the effects of long periods of experimental diabetes on this variable. Any progressive loss of sensory function might provide a convenient pharmacological model of diabetic sensory neuropathy. This study measured the latency of response to tail immersion in water maintained at 55°C ('tail flick test') in 7 rats after 6 week and 6 month periods of streptozotocin-induced diabetes and in 7 controls tested at the same ages. Results are presented as mean ± s.e. mean with p estimated using Students t-test. Blood glucose was measured (glucose oxidase method) at 6 months.

	controls (7)	diabetic (7)	
Blood glucose conc. (mM)	8.06 ± 0.69	22.39 ± 0.91	p<0.001
Latencies at 6 weeks (s)	2.60 ± 0.14	2.23 ± 0.24	NS
Latencies at 6 months (s)	2.24 ± 0.13	1.71 ± 0.14	p<0.02

These results indicate an increase in thermal sensitivity in the tails of rats with streptozotocin-induced diabetes of up to 6 months duration. The reduced latency after 6 months of diabetes might represent the hyperalgesia which has previously been observed after shorter diabetic periods. A model of diabetic neuropathy was, therefore, not demonstrated.

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375P SELECTIVE INHIBITION OF TYPE A AND B MONOAMINE OXIDASE IN ISOLATED RAT PROXIMAL CONVOLUTED TUBULES

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Epithelial cells of renal proximal tubules synthesize dopamine from L-DOPA present in the tubular filtrate and a considerable amount of the newly-formed amine has been found to be deaminated into DOPAC (Soares-da-Silva & Fernandes, 1990). Both type A and B monoamine oxidase (MAO) have been demonstrated to be present in homogenates of rat renal cortex, though MAO-A appears to be the predominant form of the enzyme (Fernandes & Soares-da-Silva, 1992). Previous work in isolated tubular epithelial cells has shown, however, that inhibition of deamination of newly-formed dopamine could be obtained only in the presence of the selective MAO-A inhibitor Ro 41-1049, the selective MAO-B inhibitor Ro 19-6327 being without effect in reducing deamination of the amine (Guimarães & Soares-da-Silva, 1993). The present work has determined type A and B MAO activities in isolated rat proximal convoluted tubules, the area of the nephron endowed with the highest ability to synthesize dopamine from L-DOPA, and tested the sensitivity of deamination of ^{3}H-5-hydroxytrytptamine (^{3}H-5-HT) and ^{14}C-ß-phenylethylamine (^{14}C-ß-PEA) to selective MAO-A and MAO-B inhibition by, respectively, Ro 41-1049 and Ro 19-6327. Suspensions of proximal convoluted tubules were obtained from male Wistar rats and MAO-A and MAO-B activities determined using, respectively, ^{3}H-5-HT (50 to 2000 µM) and ^{14}C-ß-PEA (5 to 500 µM) as preferential substrates. The reaction was stopped by the addition of 10 µl of 3 M HCl and the deamination products measured by liquid scintillation counting. The kinetics (V_{max} in nmol/mg protein/h; K_m in µM) of MAO-A and MAO-B were as follow: MAO-A, V_{max}=81±8 and K_m=160±29; MAO-B, V_{max}=54±10 and K_m=66±7. The selective MAO-A inhibitor Ro 41-1049 (10 to 500 nM) produced a concentration-dependent decrease (22% to 65% reduction) in the deamination of ^{3}H-5-HT (500 µM) [IC_{50}=81 nM (58, 113; 95% confidence limits)] without change in the deamination of ^{14}C-ß-PEA (250 µM). The selective MAO-B inhibitor Ro 19-6327 (10 to 500 nM) produced a concentration-dependent decrease (4% to 62% reduction) in the deamination of ^{14}C-ß-PEA (250 µM) [IC_{50}=122 nM (57, 257)]; deamination of ^{3}H-5-HT (500 µM) was also affected (10% to 38% reduction) by Ro 19-6327 (10 to 500 nM) [IC_{25}=329 nM (276, 393)]. In conclusion, it is shown that MAO-A represents 60% of total tubular MAO activity and Ro 41-1049 and Ro 19-6327, both up to a concentration of 250 nM, appear to be potent and selective inhibitors of, respectively, MAO-A and MAO-B.

Supported by grant number PBIC/C/CEN 1139/92 from the JNICT.
J.T.G. is on leave from the Dept. of Biochemistry, Fac. Medicine, 4200 Porto.

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376P EFFECT OF EXPERIMENTAL PAPILLARY NECROSIS ON RENAL RESPONSE TO ATRIAL NATRIURETIC PEPTIDE AND E-24.11 INHIBITION

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Endopeptidase-24.11 (E-24.11) inhibitors inhibit the metabolism of atrial natriuretic peptide (ANP) and enhance its natriuretic effects. The enzyme is abundant in the brush border of the proximal tubule; E-24.11 inhibitors may reduce degradation of filtered ANP and facilitate an intra-renal action of the peptide on papillary ANP receptors. We have examined the effect of a specific E-24.11 inhibitor candoxatrilat (Can) (Danilewicz *et al.*, 1989) on ANP-stimulated sodium excretion in an experimental rat model of papillary necrosis. Acute papillary necrosis was produced in male Wistar rats (275-300 g) using 2-bromoethylamine hydrobromide (BEA; 250 mg kg^{-1} i.v.), control animals (CON) received vehicle alone. After 48 hrs, animals were randomised to receive a) exogenous ANP 100 ng kg^{-1} min^{-1}, b) ANP 600 ng kg^{-1} min^{-1}, c) Can 3 mg kg^{-1} followed by ANP 100 ng kg^{-1} min^{-1} or d) Can 3 mg kg^{-1} alone via a femoral vein cannula. At the end of the experiment kidneys were excised and processed for [^{125}I]-rANP *in vitro* autoradiography.

Table 1. Mean basal and peak sodium excretion (µmol min^{-1}) in CON and BEA animals

		CON		BEA		*P* value
	n	basal	peak	basal	peak	
a) ANP 100 ng kg^{-1} min^{-1}	8	3.88 ± 0.70	10.10 ± 1.50	4.00 ± 0.58	9.27 ± 2.08	N.S.
b) ANP 600 ng kg^{-1} min^{-1}	6	6.75 ± 1.19	16.73 ± 1.63	5.32 ± 1.61	14.80 ± 2.14	N.S.
c) Can + ANP	10	4.09 ± 0.97	18.06 ± 2.10	2.21 ± 0.55	11.62 ± 0.88*	0.01 peak vs CON
d) Can 3 mg kg^{-1}	6	3.83 ± 0.90	8.41 ± 1.30	2.97 ± 1.56	5.75 ± 2.25	N.S.

Data analysed by ANOVA and significance assessed using Scheffes test.

The density of [^{125}I]-rANP-(1-28) binding to the papilla of BEA-treated rats was reduced by 75% compared to CON animals ($P<0.001$), with no difference in binding to glomeruli or intra-renal arteries. Glomerular filtration rate was lower in the BEA than the CON animals but did not change significantly with treatment in any group. The profile of mean blood pressure responses was similar in the CON and BEA groups. These results suggest that the papilla plays a greater role in mediating the effect of E-24.11 inhibitors on ANP-induced natriuresis than the response to ANP alone.

Danilewicz, J.C., Barclay, P.L., Barnish, I.T., Brown, D., Campbell, S.F., James, K., Samuels, G.M.R., Terrett, N.K. & Wythes, M.J. (1989). *Biochem. Biophys. Res. Commun.*, 164, 58-65.

377P RELATIONSHIP BETWEEN INHIBITION OF ANGIOTENSIN II PRODUCTION AND ACUTE RENAL FAILURE DURING ANTIHYPERTENSIVE TREATMENT IN 2K2C GOLDBLATT HYPERTENSIVE RATS

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It is recognised that in some patients with renovascular hypertension and stenosis of the renal artery, treatment with angiotensin converting enzyme inhibitors (ACEIs) normalises blood pressure but frequently leads to the development of acute renal failure. The reasons underlying this phenomenon are unclear but could be due to the generation of angiotensin II within the kidney whereby it acts at the efferent arterioles to maintain glomerular filtration pressure and filtration rate while in the periphery it causes raised vascular resistance and blood pressure. Thus, blockade of the renin-angiotensin system will not only reduce blood pressure but will also decrease glomerular filtration and lead to renal failure. Little is known of the conditions necessary to induce renal failure and the aim of this study was to use the 2K2C Goldblatt hypertensive rat to define parameters important in making filtration angiotensin II dependent in this model.
Male Wistar rats, 135-170g, were anaesthetised (N_2O/O_2/fluothane) and silver clips of 0.25 mm diameter applied on both renal arteries at their junction with the aorta. The animals were fed a normal sodium diet (0.32% Na^+ content) for four weeks and were then kept on a normal diet or given a low sodium diet (Special Diet Services, 0.05% Na^+ content) with distilled water to drink, or given a no sodium diet (I.C.N.) with distilled water for two further weeks. The animals were then placed in metabolic cages and were given by gavage, either an ACEI, captopril, 30mg·kg^{-1} every 24h or 30 mg·kg plus 10 mg·kg^{-1} every 12h after the initial dose, or a non-specific vasodilator, hydralazine, 30 mg·kg^{-1} every 12 h. During the metabolic study, systolic blood pressure were measured with a tail cuff sphygmo-manometer every 24 h and at the end of this period an arterial blood sample was taken for the measurement of plasma urea and creatinine.
Table 1:

GROUP	SYSTOLIC BLOOD PRESSURE (mmHg)				PLASMA	
	Before treatment	+24 h	+48 h	+72 h	Urea (mmol)	Creatinine (μmol)
Captopril (Normal diet, n=12)	269±9	260±9	272±11	280±8	7.9±0.5	49±2
Captopril (low sodium diet, n=6)	242±14	183±10*	191±8*	208±1*	13.3±4.5†	85±28†
Captopril (no sodium diet, n=9)	291±5	167±21*	158±19*	169±21*	44.1±8.0†	195±26†
Hydralazine (no sodium diet, n=9)	287±7	163±10*	206±7*	193±6*	7.9±0.8	57±5

* $p<0.05$ compared to pretreatment value. † compared to captopril (normal diet) using Student's 't' test.

These studies show that progressive sodium depletion in the 2K2C Goldblatt hypertensive rats leads to a greater reduction of blood pressure and an increasingly severe renal failure when the renin-angiotensin system is blocked with captopril. By contrast, reduction of blood pressure to the same extent with hydralazine didn't cause renal failure. It is possible that the sodium balance status of renovascular hypertensive patients may be an important indicator of a risk of renal failure during angiotensin converting enzyme inhibition. It remains to be determined whether the non-peptide angiotensin II antagonists act similarly in this form of hypertension.

378P RENAL EFFECTS OF THE NON-PEPTIDE ANGIOTENSIN AT_1 RECEPTOR ANTAGONIST, GR138950

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The renal effects of anti-hypertensive drugs greatly influence their therapeutic efficacy. This is particularly true for drugs which block the activity of the renin-angiotensin system since the effects of angiotensin II (AII) in the kidney are important in its role in blood pressure control. GR138950 is a novel and specific non-peptide angiotensin AT_1 receptor antagonist (Hilditch *et al.*, 1993). This study examined the effects of GR138950 on basal renal function and its ability to block renal angiotensin receptors in barbiturate-anaesthetised dogs.

As previously described (Clark *et al.*, 1993), mean aortic blood pressure (MBP) and left renal artery blood flow (RBF) were measured, and the left ureter was cannulated for the collection of urine and determination of urine output (U_V). The following were also measured or derived: glomerular filtration rate (GFR), renal vascular conductance (RVC), absolute ($U_{Na}V$) and fractional excretion of sodium (FE_{Na}), and plasma renin activity (PRA). After administration of GR138950 (1mg.kg^{-1} +10μg.kg^{-1}.min^{-1} i.v.), the effects of the antagonist alone were monitored over three consecutive 15 min periods. AII (3-1000ng.kg^{-1}.min^{-1}) was then infused directly into the renal artery (i.r.a.) for 15 min at each dose level. The effects of AII were compared with those observed in antagonist-free dogs.

Table 1: Renal effects of GR138950 (1mg.kg^{-1} +10μg.kg^{-1}.min^{-1} i.v.) in anaesthetised dogs (n=4). *P<0.05 vs saline controls.

	RVC (ml.min^{-1}.mmHg^{-1})	RBF (ml.min^{-1})	U_V (ml.min^{-1})	$U_{Na}V$ (μmol.min^{-1})	FE_{Na} (%)	GFR (ml.min^{-1})
Basal	9.7±0.8	94.3±8.2	0.31±0.08	81.8±18.1	2.2±0.4	23.8±1.4
+ 45 min	12.0±1.1*	117.0±8.9*	0.55±0.16*	137.3±28.2*	3.5±0.6*	25.1±1.3

GR138950 caused renal vasodilatation and increased urine and sodium excretion (Table 1). There was no increase in GFR but a reduction in filtration fraction was observed (-15.1±1.2%) 45 min post-dose. Basal PRA values were low (1.9±0.8ng.AI.ml^{-1}.h^{-1}) and there was no significant change in MBP or PRA 45 min after GR138950. As previously reported (Clark *et al.*, 1993), in the absence of antagonist, AII (1-100ng.kg^{-1}.min^{-1} i.r.a., n=5) had no effect on MBP, but induced dose-related decreases in RBF, RVC, U_V, $U_{Na}V$, FE_{Na} and GFR. In dogs pre-treated with GR138950, AII dose-response curves on these parameters were displaced 8 to 31-fold to the right of those obtained in antagonist-free dogs. High doses of AII (300 & 1000ng.kg^{-1}.min^{-1}) did not cause a significant pressor response in the presence of GR138950, while pressor responses were observed in the absence of antagonist.

In conclusion, this study suggests that GR138950 is an effective antagonist of the renal haemodynamic and excretory effects of endogenous and exogenous AII. These results are consistent with the potential use of GR138950 in the treatment of hypertension.

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379P Ca^{2+}-ENTRY BLOCKERS MODULATE THE INOTROPIC RESPONSE OF THE ISOLATED RAT HEART TO BENZODIAZEPINES

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Benzodiazepines evoke both positive and negative concentration-related inotropic responses in the Langendorff rat heart, i.e. diazepam a biphasic - transient negative followed by a positive - inotropic response; midazolam a positive inotropic response (Leeuwin et al., 1993). In the presence of propranolol the responses to diazepam and to midazolam are partly reversed (Leeuwin et al., 1993). This presentation describes actions of the Ca^{2+}-entry blockers verapamil (selective for slow Ca^{2+}-channels in myocardium); and flunarizine (with no perceived actions on the slow inward current in myocardium) on the inotropic responses of the isolated rat heart after exposure to diazepam or midazolam.

Hearts of pentobarbitone anaesthetized and heparinized female rats with cannulated aortae were excised and perfused with Tyrode's solution (37°C, pH 7.0). The perfusion rate was 8ml/min. Contractile force was recorded via a latex balloon filled with water, inserted into the left ventricle. After equilibration (20 min) the heart was perfused with increasing concentrations of diazepam or midazolam, with or without a Ca^{2+}-entry blocker in the perfusate.

Verapamil (10^{-8} to 10^{-6}M) induced a significant ($P<0.05$) potentiation (up to 630 ± 50%, mean ± SEM) of the positive inotropic response that was produced by diazepam, 2 x 10^{-5} to 6 x 10^{-4}M, whereas the negative inotropic response was distinctly reversed or even completely abolished. Flunarizine (10^{-9} to 10^{-6}M) opposed both the positive (maximum effect 109 ± 10% at 10^{-6} or 10^{-7}M flunarizine) and negative inotropic (to 40 ± 10% maximally at 10^{-6}M flunarizine) response elicited by diazepam, leaving the shape of the curves perfectly intact (parallel shift to the right). The concentration of 10^{-9}M did not affect the positive inotropic response significantly ($P>0.05$), [maximum effect 130 ± 10% as compared to 140 ± 25% at 1 x 10^{-4}M diazepam], whereas the negative response was still plainly reversed at that concentration. In the presence of verapamil, 10^{-9} to 10^{-7}M, the increase in heart contractile force produced by 2 x 10^{-5} to 6 x 10^{-4}M midazolam was significantly enforced (to 199 ± 20% as compared to 52 ± 12%). At the same time the concentration-effect curves were shifted to the right, i.e. maximum effects occur at higher concentrations of midazolam, indicating antagonism. In the presence of flunarizine, 10^{-9} to 10^{-6}M, the positive inotropic response to midazolam was as well parallel shifted to the right.

In conclusion: the results suggest possible involvement of Ca^{2+}-channels in the myocardium with the response of the isolated heart on exposure to benzodiazepines.

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380P THE ANTIARRHYTHMIC EFFECT OF ISCHAEMIC PRECONDITIONING IS NOT PREVENTED BY INHIBITION OF ENDOTHELIUM-DERIVED NITRIC OXIDE IN ANAESTHETIZED RATS

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Ischaemic preconditioning has clearly been shown to reduce the severity of ventricular arrhythmias which are induced by a subsequent and sustained period of regional ischaemia. This protection is attenuated by L-NAME in dogs (Vegh et al., 1992), but the NO donor SIN-1 fails to show an antiarrhythmic effect in rats (Coker & Barnes, 1993). The purpose of this experiment was to determine whether the antiarrhythmic effect of preconditioning in rats involves the release of endothelium-derived nitric oxide (NO) by assessing the effects of L-NAME and methylene blue on this protection. The ability of NO to decrease arrhythmias was determined by assessing the effect of the NO donor, C87-3754 (Bohn et al., 1991) on arrhythmias without preconditioning.

Pentobarbitone-anesthetized male Sprague-Dawley rats were preconditioned by a 3min occlusion of the left main coronary artery followed by a 10min reperfusion, prior to a prolonged 30min occlusion. Before the preconditioning stimulus, animals received a 10min intravenous infusion of L-NAME, methylene blue or vehicle. Non-preconditioned rats received the same pretreatment, but were subjected to only a 30min control occlusion. A further group of non-preconditioning rats also received C87-3754. The ventricular arrhythmias that occurred during the prolonged occlusion were analysed.

***Table 1**: Effects of NO inhibitors and nitric oxide donor on the severity of ventricular arrhythmias in rats with or without preconditioning.*

Treatment	n	VEB's (n)	Ventricular Tachycardia Incidence (%)	Ventricular Tachycardia Number (n)	Ventricular Tachycardia %VEB's	Ventricular Fibrillation Incidence (%)	Ventricular Fibrillation Duration (s)	Mortality (%)
Occlusion only:								
Saline	16	1046±196	100 (16/16)	849±231	81	75 (12/16)	52±17	56 (9/16)
L-NAME 100mgkg^{-1}	10	600±202	100 (10/10)	373±181	68	50 (5/10)	—	40 (4/10)
MB 10mgkg^{-1}	14	821±221	93 (13/14)	474±130	58	43 (6/14)	47±12	13 (1/14)*
C87-3754 1mgkg^{-1}	10	668±184	100 (10/10)	382±142	57	60 (6/10)	—	50 (5/10)
Preconditioning:								
Saline	14	76±44***	50 (7/14)	48±10***	63	7 (1/14)***	—	7 (1/14)**
L-NAME 100mgkg^{-1}	10	110±41***	60 (6/10)	60±29***	55	0 (0/10)***	—	0 (0/10)**
MB 10mgkg^{-1}	10	52±26***	40 (4/10)	31±20***	60	10 (1/10)**	—	10 (1/10)**

Values are expressed as mean±s.e.m. in rats survived during 30min occlusion. *P<0.05, **P<0.01, ***P<0.001 *v* occlusion only saline group. Mann-Whitney or Fisher's exact.

Ischaemic preconditioning reduced significantly the severity of ventricular arrhythmias. In contrast to dogs, NO appears not to be an important mediator of preconditioning in rats with respect to arrhythmias since L-NAME and methylene blue both failed to reverse this protection. Furthermore, the failure of the NO donor to reduce ischaemia-induced arrhythmias suggests that exogenous NO does not act as a cardioprotective agent in rats.

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381P FAILURE OF SODIUM NITROPRUSSIDE TO PREVENT REPERFUSION-INDUCED VENTRICULAR FIBRILLATION FOLLOWING BRIEF ISCHAEMIA IN RAT ISOLATED HEART

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Following a sustained period (60 min) of cardiac ischaemia, N^G-nitro-L-arginine methyl ester (L-NAME) elicits an L-arginine-reversible facilitation of reperfusion-induced ventricular fibrillation (VF) (Pabla & Curtis, 1993). This suggests that nitric oxide (NO) may function as an endogenous antifibrillatory cardioprotectant (contributing to the low susceptibility to VF) following sustained ischaemia. The aim of the present study was to test the time dependence of the effects of NO by examining whether an NO donor, sodium nitroprusside (SNP) can protect the heart reperfused after a brief duration of ischaemia, at which time hearts are highly susceptible to reperfusion-induced VF (Curtis and Hearse, 1989).

Male Wistar rats (240-260g) were anaesthetised with pentobarbitone (60 mg kg^{-1}, i.p) and heparinised with 250 i.u sodium heparin (i.p). Hearts were excised and perfused (Langendorff mode) with solution containing (in mM) NaCl 118.5, $NaHCO_3$ 25.0, KCl 4.0, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.4 and glucose 11.1, pH 7.4, 37°C. Hearts (n=48) were randomised to 0 (control), 0.1, 1.0 or 10 μM SNP (n=12/group). Left regional ischaemia was induced by coronary ligation and maintained for 10 min, whereupon reperfusion-induced VF was detected using a unipolar electrogram. Coronary flow was measured by timed collection of coronary effluent. Reperfused tissue was quantified using disulphine blue dye, permitting recovery of flow within it per g tissue to be calculated (Curtis and Hearse, 1989). Data were recorded and analysed (by Dunnett's test for mean±s.e.mean data or Chi^2 for % incidence data) as described previously (Curtis & Hearse, 1989).

Coronary flow was increased by SNP in a concentration dependent manner, values, e.g., 1 min before ischaemia being 8.4±0.6 ml/min/g in controls and 11.2±0.5 ($p<0.05$), 12.5±0.3 ($p<0.05$) and13.6±0.5 ($p<0.05$) with increasing concentrations of SNP. Recovery of flow during reperfusion was also enhanced by SNP, values (ml/min/g reperfused tissue) in the above groups 1 min after the start of reperfusion being 8.8±1.3, 13.0±1.8 ($p<0.05$), 10.5±1.3 ($p<0.05$) and 12.7±1.2 ($p<0.05$) respectively. These effects are explicable by and consistent with NO release (Feelisch & Noack, 1987). SNP had no antifibrillatory activity at any of the concentrations studied (%VF being 75%, 75%, 100% and 83% in control, 0.1, 1.0 and 10 μM SNP groups, respectively). SNP had no effects on QT interval or PR interval during ischaemia (values, e.g., 5 min before reperfusion being 83±4 and 38±2 msec in controls and 78±3 and 37±1 msec with the highest concentration of SNP, respectively).

In conclusion, augmentation of cardiac NO levels by SNP, as evidenced by a large increase in coronary flow, is insufficient to prevent reperfusion-induced VF in hearts reperfused after a brief duration of ischaemia. The relevance of NO as an antifibrillatory cardioprotectant may therefore be time-dependent, becoming more substantial with sustained ischaemia.

(R. Pabla is a recipient of a Wellcome Prize Studentship from the Wellcome Trust)

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382P EFFECT OF Ro 25-3562, A SELECTIVE LEUKOTRIENE B_4 RECEPTOR ANTAGONIST, ON NEUTROPHIL ACCUMULATION IN THE ISCHAEMIC/REPERFUSED RABBIT HEART *IN VITRO*

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Leukotriene B_4 (LTB_4) is a 5-lipoxygenase metabolite of arachidonic acid that promotes neutrophil adhesion, chemotaxis and subsequent diapedesis out of the vascular space (Palmblad et al., 1990). In a preliminary study, we have shown elevated cardiac concentrations of LTB_4 (as assessed by radioimmunoassay) following regional ischaemia and reperfusion using a neutrophil suspension in isolated Krebs-perfused rabbit hearts (Lad et al., 1993). These findings were consistent with experimental and clinical observations (Sasaki et al., 1988; Otsuji et al., 1988). Ro 25-3562 (2-(4-Carboxybutoxy)-6-[6-[(3,4-dihydro-4-oxo-8-propyl-2H-1-benzopyran-7-yl)oxy] hexyl1] benzenepropanoic acid) is a selective LTB_4 antagonist which reduced LTB_4-induced neutrophil shape change (IC_{50} 20-50 nM, Yagaloff & Franco, 1992). In the present study we have used Ro 25-3562 to assess the role of LTB_4 receptors in neutrophil accumulation within the ischaemic/reperfused myocardium.

In a Langendorff-perfused (40 ml min^{-1}, 37°C) rabbit heart, a short duration of coronary artery occlusion (CAO, 30 min) followed by reperfusion (30 min) with a rabbit neutrophil suspension (2 x 10^7 cells in total over 30 min) significantly increased the accumulation of neutrophils (as assessed by myeloperoxidase activity, MPO) within the area at risk (2.1±0.4 U g^{-1} tissue, n=6, $P<0.05$) compared to the normal zone (0.3±0.2 U g^{-1} tissue, n=6). Pre-treatment with Ro 25-3562 (3 μM) throughout the period of CAO and reperfusion significantly reduced the accumulation of neutrophils in the area at risk (0.8±0.4 U g^{-1} tissue, n=6, $P<0.05$) compared to the vehicle-treated group (2.1±0.4 U g^{-1} tissue, n=6) following CAO and reperfusion. A lower concentration of Ro 25-3562 (1 μM) reduced the MPO activity within the area at risk by 46% (n=6, NS) as compared to control hearts. Additionally, induction of regional ischaemia caused significant transient reductions in left ventricular developed pressure (LVDP, 83±3 mmHg 5 min post-CAO, n=6, $P<0.05$) and coronary flow (CF, 26±4 ml min^{-1} 5 min post-CAO, n=6, $P<0.05$) compared to the basal values (98±3 mmHg and 38±3 ml min^{-1}). Reperfusion of the hearts caused a partial recovery of both parameters (93±4 mmHg and 36±4 ml min^{-1}, 5 min post-reperfusion, respectively). Rabbit hearts perfused with Ro 25-3562 (1 and 3 μM) showed similar changes in LVDP and CF during CAO (LVDP of 90±3 and 91±3 mmHg and CF of 26±2 and 24±5 ml min^{-1} 5 min post-CAO, $P<0.05$) and reperfusion (LVDP of 99±5 and 102±4 mmHg and CF of 33±3 and 36±5 ml min^{-1}, 5 min post-reperfusion, respectively) as compared to the basal values (LVDP of 105±4 and 108±6 mmHg and CF of 36±4 and 37±3 ml min^{-1}).

The LTB_4 antagonist, Ro 25-3562 significantly reduced the accumulation of neutrophils following CAO and reperfusion in the rabbit isolated heart. Since Ro 25-3562 had no direct effect on the cardiac contractile function or the CF, the present study implicates a possible direct role for LTB_4 in accumulation of neutrophils into ischaemic/reperfused isolated rabbit myocardium.

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383P PROSTAGLANDIN E_1 AND PROSTAGLANDIN E_0 REDUCE INFARCT SIZE IN A RABBIT MODEL OF ACUTE MYOCARDIAL ISCHAEMIA AND REPERFUSION

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Prostaglandin E_1 (PGE_1) and its metabolite PGE_0 (13,14 dihydroPGE_1) are vasodilator prostaglandins which inhibit platelet and neutrophil function. PGE_1 also reduces myocardial infarct size after coronary artery occlusion and reperfusion in anaesthetized cats (Schror et al., 1988) and dogs (Simpson et al., 1988). Here we have investigated the effects of PGE_1 and PGE_0 on haemodynamics and infarct size in a rabbit model of myocardial ischaemia and reperfusion.

New Zealand white rabbits (2-3 kg) were premedicated with hypnorm (0.1 ml kg^{-1} containing 0.315 mg ml^{-1} fentanyl citrate and 10 mg ml^{-1} fluanisone). General anaesthesia was then induced (30 mg kg^{-1}, i.v.) and maintained with sodium pentobarbitone. After tracheotomy, the animals were ventilated with room air (36-40 strokes min^{-1}, tidal volume: 18-20 ml). A left intercostal thoracotomy was performed and the first antero-lateral branch of the left coronary artery (LAL) was occluded for 60 min followed by 2 h of reperfusion. Left ventricular pressure (LVP), mean arterial blood pressure (MAP) and heart rate (HR) were continuously recorded. Ten min prior to LAL occlusion, different groups of animals were treated with a continuous infusion of vehicle (methyl acetate), PGE_1 (1 μg kg^{-1} min^{-1} i.v.) or PGE_0 (1 μg kg^{-1} min^{-1}). At the end of the experiment, the LAL was reoccluded and Evans blue dye (4 ml of 1% w/v) injected into the left ventricle to determine the area at risk. Infarct size was determined by as percentage of the area at risk stained with nitro-blue tetrazolium (NBT; 0.5 mg ml^{-1} for 20 min).

Baseline mean values for MAP ranged from 62 ± 3 to 64 ± 3 mm Hg and for HR from 228 ± 6 to 237 ± 6 beats min^{-1}, and were not significantly different between groups. LAL occlusion did not affect MAP or HR ($n=13$, $p>0.05$). Infusion of PGE_1 (starting 10 min prior to LAL occlusion) caused an immediate reduction in MAP of approximately 30%; and MAP remained significantly reduced throughout the experiment (i.e. at 3 h MAP was 40 ± 3 mmHg; $n=7$, $p<0.05$). However, PGE_1 infusion did not cause a change in HR ($p>0.05$). Like PGE_1, infusion of PGE_0 caused an immediate and sustained reduction in MAP of approximately 30% (MAP at 3 h: 43 ± 6 mmHg), but no change in HR ($p>0.05$). The mean values for the area at risk (expressed as % of the left ventricle) ranged from 38 ± 2 to 40 ± 3 % and were not significantly different between any of the groups studied. PGE_1 or PGE_0 caused a significant reduction in infarct size (expressed as % of area at risk) from 62 ± 5% (control, $n=13$) to 40 ± 5% ($n=7$, $p<0.05$) and 36 ± 2% ($n=7$, $p<0.05$), respectively.

Thus, PGE_1 or PGE_0 cause a significant reduction in myocardial infarct size in the anaesthetized rabbit. The mechanism of action of both vasodilator prostanoids may be, in part, due to a reduction in afterload and, hence, oxygen consumption. *(This work was supported by a project grant from Schwarz Pharma AG, Monheim, Germany).*

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384P NITRIC OXIDE SELECTIVELY ENHANCES RELAXATION IN THE INTACT VENTRICLE

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Impaired myocardial relaxation may contribute to diastolic heart failure. Recent reports indicate that nitric oxide (NO) selectively hastens relaxation in isolated myocardial preparations e.g. ferret papillary muscles, isolated cardiac myocytes, via elevation of myocardial cyclic GMP (cGMP), which appears to reduce the myofilament response to Ca^{2+}. We examined the effects of NO on ventricular function in isolated ejecting ('working') guinea-pig hearts (constant loading and heart rate; recirculating Krebs buffer; 37°C) using a high-fidelity 2F micromanometer-tipped catheter to measure left ventricular pressure (LVP) and its first derivative dP/dt. LVP fall was biphasic and well characterised by two monoexponential time constants; (T_E) for early relaxation and (T_L) for late relaxation. Sodium nitroprusside was used as an exogenous source of NO and substance P to release NO endogenously from the coronary endothelium.

	Control	SNP 0.1μM	SNP 1μM	SNP 10μM	SubP 0.1μM	Nic 10nM	Hb 1μM control	SNP 1μM + Hb 1μM	SubP 0.1μM + Hb 1μM
Peak CF	1.9±1.4	10.6±1.9*	23.9±1.0*	56.2±13.2*	19.9±2.2*	35.8±1.5*	0.4±0.3	12.3±1.5†	0.7±0.7†
T_E	-0.8±1.9	-5.8±2.0	-18.6±1.4*	-15.4±3.1*	-11.3±2.4*	-0.7±7.8	0.2±3.7	-1.2±4.5†	4.2±5.0†
T_L	4.9±2.3	10.2±3.1	1.1±3.8	-1.6±5.1	8.3±3.8	26.3±8.1*	8.3±3.7	6.6±6.4	6.9±3.3
tPP	-0.5±1.0	0.3±0.5	-4.9±1.3*	-4.4±1.6*	-2.4±2.2	0.1±1.3	2.2±1.7	0.7±0.8†	3.3±0.8

Table 1: Percent changes (± s.e.mean) in peak coronary flow (CF), relaxation time constants (T_E & T_L) and time to peak pressure (tPP) after 12 mins. (SNP - sodium nitroprusside; SubP - substance P; Nic - nicardipine; Hb - human haemoglobin: *$p<0.05$ c.f. control; †$p<0.05$ c.f. same drug without Hb)

SNP and SubP both hastened the onset of and induced faster early relaxation (reduced tPP & T_E), without effecting end-diastolic LVP or 'systolic' parameters e.g. peak dP/dt or LVP (data not shown). These effects were inhibited by the NO scavenger, Hb and were not seen with the cGMP-independent vasodilator, nicardipine, although it increased CF to a similar degree. Thus, these data show that both exogenous and endogenous NO selectively enhanced relaxation in the intact LV, independently of its effect on CF. An intervention which improves relaxation without compromising contraction could be beneficial in the treatment of heart failure.

385P INHIBITION OF NITRIC OXIDE REDUCES INFARCT SIZE IN THE RABBIT ISOLATED HEART THROUGH ADENOSINE RELEASE

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Brief periods of ischaemia followed by reperfusion render the heart resistant to infarction from a subsequent ischaemic insult. This is termed ischaemic preconditioning (IP). The mechanism is not known but there is evidence that IP is triggered by adenosine acting on the A1 receptors specifically (Liu et al., 1991).We have previously reported that the protection afforded by IP was not diminished when nitric oxide (NO) synthesis was inhibited, in the *in vivo* rabbit heart (Patel et al.,1992). Furthermore, we have also shown both *in vivo* and *in vitro* that inhibition of NO synthesis in the rabbit heart prior to regional ischaemia and reperfusion significantly reduces infarct size (Patel et al., 1993).This study examined the mechanism by which inhibition of NO synthesis protects against ischaemia and reperfusion injury and whether this protection shares a common pathway with IP. To this end, we used nitro-L-arginine methyl ester (L-NAME, 30 μM) and 8-p-sulphophenyltheophylline (SPT, 75.5 μM) to block NO synthesis and adenosine receptors respectively.

New Zealand White rabbits were anaesthetised with pentobarbitone (40 mg/kg) and ventilated. Hearts were rapidly excised and Langendorff perfused. Six groups were studied; CONTROL; L-NAME; L-NAME+SPT; IP; IP+L-NAME; IP+SPT+L-NAME. The IP protocol was 5 minutes left coronary occlusion, 10 minutes reperfusion followed by 45 minutes ischaemia and 180 minutes reperfusion. At the end of each experiment the artery was re-occluded and the coronary circulation perfused with fluorescent microspheres to delineate the risk area (R). Tetrazolium was used to demarcate the infarcted area (I). I/R ratios were then calculated. Coronary effluent samples were collected following stabilisation, and then after 10 minutes treatment with L-NAME or IP. The samples were assayed for adenosine (ADO) by high performance liquid chromatography.

	CONTROL	L-NAME	SPT+L-NAME	IP	IP+L-NAME	IP+SPT+L-NAME
(n)	7	8	7	6	6	5
Risk area cm^2	3.4±0.5	3.0±0.5	3.7±0.6	3.4±0.6	3.7±0.6	4.4±0.6
Infarct/risk %	65±0.3	42±5*	58±8	22±2**	20±2**	59±3
ADO (μM)	6±2	23±4*		83±7**		

*p<0.05, ** p < 0.001 vs CONTROL and L-NAME (ANOVA)

Treatment with L-NAME or IP gave rise to an increased adenosine concentration in coronary effluent. Protection seen with both L-NAME and IP was abolished on treatment with SPT, suggesting a common mechanism. L-NAME induced less protection then IP and led to lower ADO level in the coronary effluent than IP, suggesting that a dose-response relationship may exist between the amount of ADO released and the degree of protection obtained. Thus treatment with L-NAME may have led to partial preconditioning of the myocardium.

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386P THE ROLE OF ATP-SENSITIVE POTASSIUM (K_{ATP}) CHANNELS IN CARDIAC ARRHYTHMIAS IN RAT ISOLATED HEARTS

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K_{ATP} channels are opened in cardiac myocytes by a reduced intracellular ATP concentration. This causes K^+ efflux which, whilst conceivably inhibiting after-depolarisations, might facilitate re-entrant arrhythmogenic mechanisms (Nichols & Lederer, 1991). Evidence for the role of these channels in cardiac arrhythmias is conflicting (Lynch *et al.*, 1992). This study assessed their role in Langendorff-perfused rat isolated hearts using the putative K_{ATP} opener Ro 31-6930 (Paciorek *et al.*, 1990) at 0.5 or 5 μM, the K_{ATP} blocker glibenclamide at 10 μM, or their combination.

Regional ischaemia was induced 15 min after drug addition by occluding the left main coronary artery (LMA) for 10 min. This caused a discreet region of non-perfused muscle, assessed by staining, of 52±3% (mean ± s.e, n=10) total ventricular weight. The cardiac electrogram was used to quantify ventricular tachyarrhythmias during ischaemia and 10 min reperfusion. The incidences of arrhythmias were compared using a χ^2 test (with Yates's Correction) and their durations were compared using a Mann Whitney test. A P value of less than 0.05 was considered to be significant.

During ischaemia, in a control group using Tyrode solution at 37.5°C (n=10), the incidence of ventricular tachycardia (VT) was 10% and its duration was 0.3±0.3 s. Ro 31-6930 at 0.5 μM (n=10), glibenclamide at 10 μM (n=9) and their combination (n=10) had no effect on the incidence or duration of VT. Ro 31-6930 at 5 μM (n=9) increased the incidence of VT to 89% (P<0.001). In this group VT onset occurred within 4 min of LMA occlusion in 67% of hearts and its duration was 340±58 s. The combination of 5 μM Ro 31-6930 and 10 μM glibenclamide (n=10) restored the incidence and duration of VT to control.

During reperfusion, in the control group (n=10), the incidence of VT was 90% and its duration was 331±84 s. Groups with Ro 31-6930 at 5 μM (n=9), glibenclamide at 10 μM (n=9) and their combination (n=10) exhibited a similar incidence and duration of VT to the control. Ro 31-6930 at 0.5 μM (n=10) reduced the incidence of VT to 20% (P<0.001). The combination of 0.5 μM Ro 31-6930 and 10 μM glibenclamide (n=10) reduced the incidence of VT to 50% (P=0.02) and its duration to 17±6 s.

In conclusion, the putative K_{ATP} channel opener Ro 31-6930 had opposing effects at different concentrations. Ro 31-6930 had anti-arrhythmic activity at 0.5 μM during post-ischaemic reperfusion and pro-arrhythmic activity at 5 μM during ischaemia. The K_{ATP} channel blocker glibenclamide at 10 μM antagonised the pro-arrhythmic effect during ischaemia, but incompletely offset the anti-arrhythmic effect of Ro 31-6930 during reperfusion.

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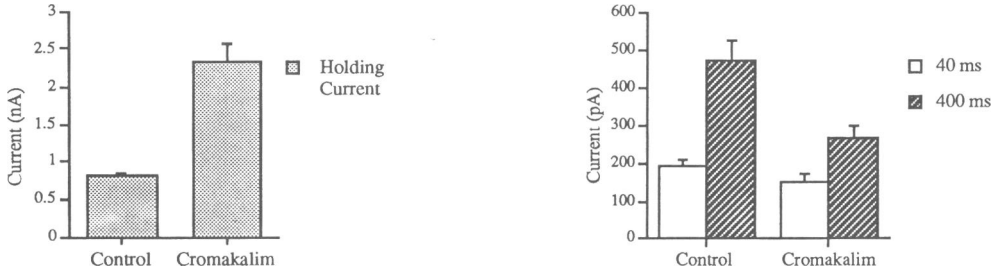
387P PARALLEL ACTIVATION OF K_{ATP} AND REDUCTION OF DELAYED RECTIFIER CURRENT BY CROMAKALIM IN GUINEA-PIG VENTRICULAR CARDIAC MYOCYTES

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The possible changes in components of the delayed rectifier potassium current (see Sanguinetti and Jurkiewicz 1990) when the ATP-dependent potassium (K_{ATP}) current was activated by cromakalim were investigated in cardiac ventricular muscle.

Single cells were isolated from guinea pig ventricular muscle. The delayed rectifier potassium current was studied under voltage clamp conditions (balanced salt solution with 2.5 mM Ca^{2+} at 36°C; microelectrodes containing 20 mM BAPTA to suppress the cytosolic Ca^{2+} transient) and measured as an outward tail current upon repolarization to a holding potential of -40 mV following depolarizations to +40 mV. The current at 40 ms was taken as a measure of the early component and that at 400 ms as a measure of the late component.

The figure shows that after 5.5 minutes exposure to 20 μM cromakalim, the holding current increased significantly from 0.8 ± 0.05 nA to 2.33 ± 0.25 nA ($p<0.001$; n=9) consistent with activation of the K_{ATP} current. Over the same time course, the amplitude of both the early and late components of the delayed rectifier potassium current were significantly reduced. The tail current at 40 ms was reduced from 191 ± 18 pA to 151 ± 19 pA ($p<0.01$; n=9) and at 400 ms, from 478 ± 51 pA to 266 ± 32 pA ($p<0.001$; n=9). There was no significant change in holding current or in either components of the delayed rectifier after exposure to DMSO vehicle (n=4).



The parallel block of delayed rectifier current and activation of K_{ATP} current may be separate effects of cromakalim. The observations are also consistent with the recent intriguing hypothesis that cromakalim may lead to a conversion of delayed rectifier potassium channels into K_{ATP} channels (Edwards *et al,* 1993).

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This work was supported by the British Heart Foundation.

388P FURTHER INVESTIGATIONS OF THE PREJUNCTIONAL α_2-ADRENOCEPTORS OF RAT ATRIUM

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We have previously demonstrated that the prejunctional α_2-adrenoceptor of rat atrium is distinct from the prejunctional $\alpha_{2A/D}$-adrenoceptor of rat vas deferens, and resembles the α_{2B}-ligand binding site of rat kidney (Smith & Docherty, 1992). Recently, other authors have suggested that the prejunctional α_2-adrenoceptor of rat atrium does resemble the $\alpha_{2A/D}$ site (Limberger et al., 1992). Hence, we have carried out further investigations of the prejunctional α_2-adrenoceptor of rat atrium.

Antagonist potency prejunctionally in rat atrium was expressed as an EC_{30} (concentration producing 30% increase in the electrical stimulation-evoked release of tritium in tissues pre-incubated with ^{3}H-noradrenaline). Ligand affinity was expressed as a K_i for the displacement of ^{3}H-yohimbine binding to membranes of rat kidney (α_{2B}) and submandibular gland ($\alpha_{2A/D}$). Antagonists employed included yohimbine, chlorpromazine, WB 4101, BDF 8933, prazosin, ARC 239, BRL 44408 and benoxathian (see Smith & Docherty, 1992).

The antagonists WB 4101 and benoxathian, which had low affinity for the $\alpha_{2A/D}$ ligand binding site of rat submandibular gland (-log Ki values of 6.38±0.33 and 5.63±0.11, respectively), had high potency prejunctionally in rat atrium (-log EC_{30} values of 7.70±0.22 and 6.44±0.23, respectively). The correlation between potency at the functional prejunctional α_2-adrenoceptor of rat atrium and affinity for the $\alpha_{2A/D}$ ligand binding site of rat submandibular gland was relatively poor ($r=0.69$, $P<0.01$, n=14), whereas the correlation with affinity for the α_{2B} ligand binding site of rat kidney was good ($r=0.92$, $P<0.001$, n=14). However, there was also a relatively good correlation with published data obtained at the α_{2C}-ligand binding site (Uhlen et al., 1992)($r=0.87$, $P>0.001$, n=11). For the 8 antagonists in common (listed above), the correlations with the functional prejunctional α_2-adrenoceptor in rat atrium were $r=0.66$ (N.S.), $r=0.95$ ($P<0.001$) and $r=0.86$ ($P<0.01$), for the $\alpha_{2A/D}$-, α_{2B}-and α_{2C}-sites, respectively.

It is concluded that the prejunctional α_2-adrenoceptor of rat atrium is not an $\alpha_{2A/D}$ site, resembles the α_{2B}-site, but may be an α_{2C}-site as found in central nervous system (Uhlen et al., 1992) .

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389P DIFFERENT INOTROPIC RESPONSIVENESS IN HUMAN ATRIAL AND VENTRICULAR TRABECULAE

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In a previous study, we found that 5-HT increased the force of contraction in the atrial but not in ventricular tissue (Schoemaker *et al.*, 1993). In the present study, we report the inotropic effects of human calcitonin gene-related peptide (CGRP), angiotensin II (AII), adenosine, somatostatin and acetylcholine (ACh) in atrial and ventricular trabeculae from the same heart.

Right atrial and left ventricular trabeculae (< 1 mm thickness) from human non-diseased hearts were carefully dissected free and mounted in organ baths (37 °C, gassed with 95% O_2 and 5% CO_2 and paced at 1 Hz) for isometric tension measurement. Noradrenaline (NA), which was used to check the contractile responsiveness of the tissues, increased contractile force in both atrial and ventricular tissues. CGRP (10^{-9} to 10^{-7} M) or AII (10^{-9} to 10^{-5} M) increased contractile force in human atrial trabeculae up to 36±8% (n=8) and 42±8% (n=4) of the response to 10^{-5} M NA, respectively. Similar to 5-HT, no effect of CGRP and AII was found in human ventricular trabeculae. Adenosine (10^{-8} to 10^{-5} M) and somatostatin (10^{-8} to 10^{-6} M) caused a negative inotropic effect on baseline atrial contractility (down to 54±17% and 51±25%, n=5, respectively). No response was detected on baseline ventricular contractility. Adenosine, but not somatostatin, reduced the contractile force after pre-stimulation with 10^{-5} M NA in atrial tissue as well as in ventricular tissue (down to 52±12% and 81±6%, at 10^{-5} M, n=6, respectively). ACh caused a more complex effect; in atrial tissue, ACh exhibited a biphasic concentration response curve, consisting of an initial negative inotropic response (at 10^{-9} to 10^{-7} M, from 120±41 mg at baseline, down to 48±16 mg, n=8), followed by a positive inotropic response (at 10^{-6} to 10^{-3} M, from 48±16 mg to 77±15 mg at 10^{-7} M, n=8). On ventricular baseline contractility, ACh (10^{-9} to 10^{-4} M) induced only a positive inotropic effect, starting at 10^{-9} M (from 252±65 up to 353±71 mg, n=6). After pre-stimulation with 10^{-5} M NA, ACh reduced the contractile force in both atrial (down to 14±4%, n=7) and ventricular (down to 61±5% at 10^{-3} M, n=6) tissue.

Data from the present study show that, except for NA, the atrium displays a stronger response than the ventricle and can be affected by either positive or negative inotropic agents. Ventricular tissue only responds to positive inotropic agents. Negative inotropic effects (adenosine and acetylcholine) were only seen in pre-stimulated trabeculae. These results may provide a better understanding of the physiology of atrial and ventricular contractility, as well as an important direction for drug development.

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390P 4-ACETAMIDE-4'-ISOTHIOCYANATOSTILBENE-2,2'-DISULPHONIC ACID INHIBITS STRETCH-INDUCED VENTRICULAR PREMATURE CONTRACTIONS IN ISOLATED GUINEA-PIG HEARTS

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4-Acetamide-4'-isothiocyanatostilbene-2,2'-disulphonic acid (SITS), a stretch-activated Cl^- channel blocker, can reduce stretch activated anion current, which relates to the modification of the cardiac pacemaker activity in rabbit (Hagiwara, et al., 1991). Also, previous experiments have shown that manipulation of anion homeostasis can reduce ventricular arrhythmias in Wistar rats (Ridley & Curtis, 1992). We tested the hypothesis that SITS may be an inhibitor of stretch-induced arrhythmias, by stretching isolated guinea-pig hearts perfused by the Langendorff apparatus.

Guinea-pig hearts were perfused with oxygenated (95%0_2, and 5%CO_2) modified Krebs-Henseleit solutions (n=12), followed by solutions containing SITS 0.1 mM (n=7). We increased ventricular loading suddenly via a saline-filled latex balloon in the left ventricle. The degree of inflation was determined by the volume that triggered the most ventricular premature contractions (VPCs), the volume being kept within physiological limits. We recorded monophasic action potentials from the epicardium using suction electrodes. SITS significantly inhibited stretch-induced ventricular premature contractions (P<0.001, Wilcoxon signed rank test) (figure 1).

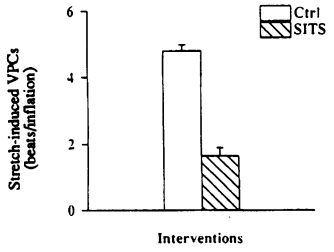


Fig 1. Stretch-induced VPCs in control (ctrl) and with 0.1 mM SITS (Mean +/- SEM).

The data suggest that Cl^- currents are involved in the membrane stretch, and these membrane currents relate to normal and abnormal cardiac pacemaker activity, and that stretch-activated anion channels as well as stretch-activated cation channels (Zhou, et al. 1993) contribute to the genesis of stretch-induced ventricular arrhythmias in guinea-pig hearts.

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391P INTERACTIONS OF ZENECA ZD7288, A NOVEL SINO-ATRIAL NODE FUNCTION MODULATOR, WITH BILATERAL STELLATE NERVE STIMULATION IN THE ANAESTHETISED DOG

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ZD7288 is a novel member of a pharmacological class of compounds which lower heart rate (HR) by direct action on the sino-atrial node (SAN) (BoSmith *et al.*, 1993). ZD7288 has been shown to reduce HR in anaesthetised dogs without depressing myocardial function (Rouse & Johnson, 1993,). Alinidine has a mode of action on the SAN in common with ZD7288 (BoSmith *et al.*, 1993), and has been shown not to affect HR increases to stellate nerve stimulation (Hageman et al., 1985).

Randomised, duplicated responses to bilateral stellate nerve stimulation (0.2 to 10 Hz, 2 ms pulses, supramaximal) in pentobarbitone-anaesthetised dogs (n=3) during mid-sternal thoracotomy, were determined before and after ZD7288 (0.05 mg.kg^{-1} i.v.) and atenolol (0.03 mg.kg^{-1}i.v.) administration. Aortic pressure, stroke volume and blood gases were monitored. Measurements were made immediately before stimulation and during peak responses of heart rate (HR) and contractile function, both as dP_{LV}/dt and right ventricular cardiac contractile force (CCF_{RV}, using a strain gauge arch sutured to the ventricular free wall). ZD7288 reduced intrinsic HR from 128.1 ± 3.2 to 96.7 ± 4.1 beats.min^{-1}, ($P<0.05$, Student's group t-test). dP_{LV}/dt changed from 2393 ± 343 to 2178 ± 309 mmHg.s^{-1}, (NS) and CCF_{RV} from 30.4 ± 5.1 to 22.6 ± 3.1 gm, (NS). ZD7288 non-competitively reduced HR increases in reponse to stellate stimulation by an average of approximately 28% ($P<0.05$) whilst dP_{LV}/dt responses were not affected (figure 1). The same result was evident for CCF_{RV}. Atenolol, 0.03 mg.kg^{-1} i.v., given after ZD7288 antagonised both HR and contractile responses in roughly equal proportions (figure 1), but caused no further decrease in HR or contractility without stimulation.

We conclude that ZD7288, by reducing sympathetically mediated changes in HR as well as intrinsic HR, without affecting sympathetically mediated contractile function reponsiveness, should be of benefit in ischaemic heart disease, particularly when contractile function may be compromised.

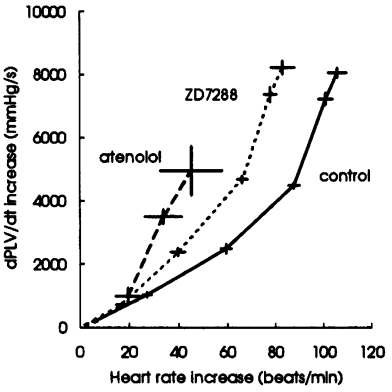


Figure 1 Heart rate:contractile function relationships during stellate stimulation at 0.2, 0.5 1, 2, 5 & 10 Hz (2, 5 & 10 Hz for atenolol).

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392P [^{123}I]4-IODODEXETIMIDE AS A RADIOLIGAND FOR THE IMAGING OF CARDIAC MUSCARINIC RECEPTORS

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The human heart is strongly influenced by the parasympathetic system. In several cardiac disorders, such as cardiac failure and ischaemic heart disease, the influence of the parasympathetic system is blunted, possibly leading to arrhythmia. The heart contains predominantly M_2-subtype selective muscarinic receptors. Whether the binding site itself or the effector pathway may be impaired remains subject to debate. Mukherjee et al. (1979) observed no change in muscarinic receptor density in canine left ventricular tissue after ischaemia, but they did not relate this finding to changes in functional responses to muscarinic agonists. Vatner et al. (1985, 1988) reported a reduction in cardiac muscarinic receptor density in dogs with left ventricular failure. The present study was designed to image muscarinic receptors in vivo. For this purpose we intend to develop suitable radioligands. [^{123}I]4-Iododexetimide (IDEX) has successfully been used to image muscarinic receptors in human brain using SPECT (Single Photon Emission Computerised Tomography). A major advantage of the use of IDEX as a radioligand is the availability of the inactive but labelled enantiomer [^{125}I]-Levetimide that can be used for the determination of the non-specific binding.

In the present study we tested, *in vitro*, the affinity of (IDEX) for the cardiac muscarinic receptor. For [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) displacement studies, left ventricular tissue of male Wistar rats (weight 250-300g) was homogenised and incubated for 20 minutes with HEPES buffer (total binding), 10μM dexetimide (non-specific binding) or the displacer. Specific binding was determined as the difference between total binding and non-specific binding. Separate assays were performed in duplicate. We compared the affinity of dexetimide (DEX) for the muscarinic receptor with that of IDEX and with a well known M_2-subtype selective muscarinic antagonist, AFDX 116, and with gallamine.

With respect to pD_2 values we established the following rank order of potency: DEX (pK_i=7.7 ± 0.24, n=4), AFDX 116 (pK_i=6.2 ± 0.18, n=6), gallamine (pK_i=5.4± 0.75, n=6). The iodination of dexetimide did not affect the affinity for the receptor (pK_i=7.7 ± 0.32, n=4).

Accordingly, [^{123}I]4-Iododexetimide appears to be an effective displacing agent. Its characteristics are preserved after iodination. Concomitantly, [^{123}I]4-Iododexetimide may be considered as a potentially useful tool for SPECT-imaging of cardiac muscarinic receptors in vivo in humans.

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393P DIFFERENCES IN TRANSCRIPTION AND TRANSLATION OF LONG AND SHORT $G_{S\alpha}$, THE STIMULATORY GUANINE NUCLEOTIDE BINDING PROTEIN α-SUBUNIT IN HUMAN ATRIUM

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We have previously reported the relative mRNA and protein levels of the long and short forms of Gsα (GsαL & GsαS) in human atrium (Ferro *et al.*, 1993; Jia *et al.*, 1993). We have now measured the relative proportions of the serine$^+$ (ser$^+$) and serine$^-$ (ser$^-$) variants of GsαL and GsαS in human atrium, and assessed, indirectly, whether their differential expression may *[i]* regulate post-translational modifications, such as phosphorylation, of Gsα , and *[ii]* itself be regulated by intracellular cyclic AMP levels.

Right atrial strips (~150 mg) were obtained during cardiac bypass operations at Papworth Hospital, and either homogenized in guanidinium isothiocyanate for RNA extraction or the membranes solubilised for isoelectric focussing and SDS PAGE of the protein. After reverse transcription, GsαL and GsαS cDNA's were co-amplified by polymerase chain reaction (PCR) using primers spanning the variably spliced exon 3, and purified by agarose gel and 'geneclean'. The ser$^+$ and ser$^-$ variants were then estimated by Single Nucleotide Primer Extension (SNUPE): one round of PCR was performed with a primer whose 3' base preceded the first residue to differ between ser$^+$ and ser$^-$ variants, and a single [^{32}P]-deoxynucleotide, dCTP or dTTP, was incorporated specifically into the ser$^+$ and ser$^-$ variants, respectively. Autoradiograms of a sequencing gel of the resulting [^{32}P]-labelled primer were analysed by ImageQuant (Molecular Dynamics). Cloned ser$^+$ and ser$^-$ cDNA's were used as positive controls. For separate visualisation of the proteins, 2-D gels of the solubilised membranes were immunoblotted with an anti-Gsα antiserum kindly donated by Dr. G. Milligan.

The initial PCR amplification with common primers for GsαL and GsαS showed similar expression of these. SNUPE was semi-quantitative, with signals linearly related to dilution of cDNA within an assay, but low reproducibility between assays. Therefore equal numbers of atria from 15 β-blocked and 16 non β-blocked patients were assayed together. The ratio of ser$^-$ to ser$^+$ variants was 53.8 ± 13.5 for GsαL, compared to 2.91 ± 1.02 for GsαS ($p<0.001$), with no significant difference in the two groups of patients. Since this serine is a potential phosphorylation site we compared the iso-electric points of GsαL and GsαS in the atria of 4 β-blocked and 4 non-β-blocked patients. 2-D gels gave a similar pattern in both groups of patients, with the pI values of the short residues (pI 5.18 ± 0.26) being more acid than of the long (5.87 ± 0.19) ($p<0.01$). As in our previous quantitative studies of total Gsα mRNA and protein levels, immunoblotting showed more total GsαL than GsαS.

All four Gsα variants are expressed in human atrium, but GsαL is almost entirely of the ser$^-$ form. The greater stability of GsαL suggested by its protein/mRNA ratio together with its lesser modification by acidic residues point to the serine as a possible key residue in post-translational changes and degradation of Gsα. Although cAMP directed phosphorylation of Gsα occurs *in vitro* (Pyne *et al.*, 1993), the lack of marked difference in any of our results between β-blocked and non-β-blocked patients suggests indirectly that cAMP is not the principal regulator *in vivo* of transcriptional or post-translational modifications of Gsα.

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394P DIFFERENTIAL EFFECTS OF NOVEL CARDIOTONIC AGENTS ON THE VENTRICULAR ACTION POTENTIAL

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Several cardiotonic agents, which produce positive inotropic and vasodilator effects, have been developed for the treatment of congestive heart failure. Of these drugs, the selective phosphodiesterase (PDE) inhibitors, such as enoximone, have received considerable attention. More recently the mechanism of the inotropic action of flosequinan has been attributed to such PDE inhibition (Yates, 1991). The present study compared the actions of selective (enoximone, flosequinan, sulmazole, rolipram) and non-selective (IBMX) PDE inhibitors, and examined the combined action of flosequinan and the PDE IV inhibitor, rolipram, on the ventricular action potential. Left ventricular papillary muscles were isolated from male Sprague-Dawley rats (200-250g) and the tissue was stimulated supramaximally using 1 ms pulses at 1 Hz. Conventional intracellular microelectrode techniques were used to record action potentials which were digitized and stored on computer for subsequent analysis. The maximal upstroke velocity (V_{max}) of the action potential (AP) was obtained from the differential. Comparisons were made using analysis of variance followed by a multiple range (Dunnet's) or paired Students 't' test. Data are presented as the mean±standard error, n=5.

Enoximone (10^{-5}M to 3×10^{-4}M) had no effect on parameters of the AP. Sulmazole, at a concentration of 3×10^{-4}M, produced a significant decrease ($p<0.05$) in the duration of the AP, 9.6±1.0 and 17.2±1.7 ms, at 50% and 75% repolarization, whereas predrug values were 13.9±0.9 and 24.6±1.8 ms, respectively. This effect was attenuated at a concentration of 10^{-3}M and was accompanied by decreases ($p<0.05$) in V_{max} and amplitude to 124±6 V.s^{-1} and 93.7±2 mV, respectively, from predrug values of 151±7 V.s^{-1} and 103.1±1.3 mV, respectively. On the other hand, rolipram increased ($p<0.05$) the duration of the AP, but only at elevated concentrations ($>10^{-4}$M); the maximal response observed at 6×10^{-4}M was 26.6±2.6 and 59.4±9 ms at 50% and 75% repolarization whereas the predrug values were 14.5±0.9 and 23.8±1.3, respectively. This prolongation was accompanied by decreases in amplitude and V_{max} to 89.6±0.9 mV and 70±10 V.s^{-1} from predrug values of 101±0.6 mV and 157±16 V.s^{-1}, respectively. The resting membrane potential did not change throughout the study (range: -79.8±1.8 to -83.1±1.2 mV). IBMX increased the duration of the AP in a concentration-dependent manner over the range 10^{-5}M to 10^{-3}M ($p<0.05$), and EC_{50} values were 0.94±0.09 and 1.1±0.1 ms at 75% and 50% repolarization, respectively. Flosequinan did not have any effect on the AP duration, which at 75% repolarization was 19.6±1.3 ms. However, the combination of flosequinan and rolipram (3×10^{-4}M) (44±8 ms) produced a greater prolongation ($p<0.05$) of the AP than that produced by rolipram alone (33±5 ms).

In contrast to the non-selective PDE inhibitor, IBMX, it is likely that enoximone and flosequinan had little effect on I_{Ca}. Rolipram prolonged the duration of the AP at elevated concentrations in accordance with an action on accumulation of cyclic AMP, which is consistent with the depressed V_{max}. The potentiation of the rolipram response, in terms of prolongation of the AP duration, could be based on a novel mechanism of action, such as an enhanced intracellular release of Ca^{2+} by flosequinan.

This work was supported by the British Heart Foundation

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395P HAEMODYNAMIC EFFECTS OF OUABAIN IN THE ANAESTHETISED MINI-PIG: PRESSURE-VOLUME ANALYSIS

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The administration of Ouabain to the healthy animal or man causes little change- or even a fall- in stroke volume. This has been attributed to a marked increase in afterload in conjunction with only a moderate inotropic effect (see Vatner et al., 1971). A fall in venous return might also contribute to the reduction in stroke volume (Ross et al., 1960). Still further, this preload reduction may have lead to an underestimation of the inotropic effect as assessed by LV dp/dt_{max}, an index known to be preload-dependent. In order to clarify the mechanism of action of ouabain we used left ventricular (LV) pressure-volume (PV) analysis (Sagawa, 1981), an approach providing a relatively load-independent index of contractility. Male Göttinger mini-pigs (13.8 - 14.8 kg) were anaesthetised (pentobarbital) and ventilated. Indices of preload (end-diastolic volume; EDV), afterload (arterial elastance; E_a) and LV contractility (end-systolic elastance; E_{es}), as well as stroke volume (SV), were obtained from PV loops. In addition, heart rate (HR), cardiac output (CO), mean arterial blood pressure (MABP) and ejection fraction (EF) were determined at baseline and 10, 20 and 30 min following i.v. bolus doses of Ouabain (10- 30 $\mu g\ kg^{-1}$) or saline vehicle.

Ouabain ($\mu g\ kg^{-1}$)	Time min	E_{es} mmHg ml^{-1}	E_a mmHg ml^{-1}	EDV ml	SV ml	HR bpm	CO l min^{-1}	MABP mmHg	EF %
baseline	0	7.1 ± 0.8	11.6 ± 1.0	25.7 ± 2.3	11.5 ± 0.9	104 ± 5	1.19 ± .09	108 ± 6	45.0 ± 2.7
10	10	7.4 ± 0.8	12.6 ± 1.1**	25.7 ± 2.2	11.4 ± 0.9	103 ± 5	1.17 ± .09	116 ± 6***	44.8 ± 2.9
	20	7.6 ± 0.9	12.2 ± 1.1	24.8 ± 2.2	11.4 ± 0.9	102 ± 5	1.15 ± .09	112 ± 6**	46.2 ± 2.9
	30	7.6 ± 0.8	12.0 ± 1.1	24.5 ± 2.3	11.3 ± 0.9	102 ± 5	1.14 ± .09	110 ± 6	46.8 ± 2.3*
	40	9.2 ± 0.9*	13.5 ± 1.0**	25.5 ± 2.4	11.5 ± 0.8	102 ± 5	1.16 ± .09	123 ± 7***	45.7 ± 3.0
	50	9.8 ± 0.9**	12.8 ± 0.9	23.5 ± 2.3*	11.4 ± 0.9	101 ± 5	1.15 ± .10	118 ± 6**	49.0 ± 3.2**
	60	9.9 ± 0.8**	12.2 ± 0.9	22.6 ± 2.2**	11.6 ± 1.0	100 ± 4	1.15 ± .11	114 ± 6	51.7 ± 3.1***
30	70	11.6 ± 1.4*	14.4 ± 1.4*	23.6 ± 2.2	11.4 ± 1.0	102 ± 4	1.18 ± .11	140 ± 3***	48.3 ± 3.0
	80	12.8 ± 1.4**	13.3 ± 1.2	20.3 ± 2.2*	11.2 ± 1.2	103 ± 3	1.16 ± .13	129 ± 3**	54.8 ± 3.1*
	90	14.5 ± 1.1***	13.1 ± 0.9	17.3 ± 2.0***	10.6 ± 1.0	102 ± 4	1.07 ± .10	117 ± 2	61.1 ± 3.4***

Data are shown as the mean ± s.e. mean of 8 animals, with * P < 0.05;**P < 0.01, ***P < 0.001 compared to baseline

Ouabain caused a marked and dose-dependent increase in contractility (E_{es}), and ejection fraction (EF) without changing stroke volume (SV). This lack of effect on stroke volume was due both to an initial transient increase in afterload (E_a) and a delayed reduction in preload (EDV). The increase in MABP was secondary to the increase in afterload. In conclusion, in contrast to previous studies, Ouabain was shown to cause a marked increase in contractility as assessed by E_{es}. However, as a consequence of changes in afterload as well as preload, this increase in contractility was not manifested as an increase in stroke volume or cardiac output.

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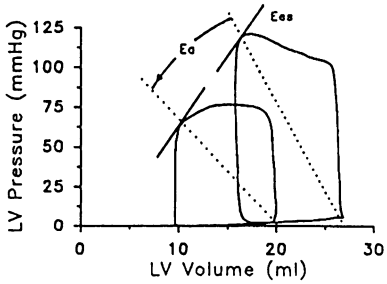
396P HAEMODYNAMIC EFFECTS OF THE NO DONOR SIN-1 IN THE ANAESTHETISED GUINEA-PIG: QUANTIFICATION OF MYOCARDIAL OXYGEN CONSUMPTION BY PRESSURE-VOLUME AREA

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Molsidomine, an NO-donor used in the treatment of angina, lowers left ventricular end-diastolic pressure and systemic vascular resistance (Bassenge, 1989). Based on these observations, it has been speculated that one of the beneficial effects of Molsidomine is a reduction in myocardial oxygen demand due to a reduction of preload, afterload, or both. The present study was designed to quantify, as well as elucidate the mechanism underlying, the reduction of oxygen demand following SIN-1, the active metabolite of Molsidomine. Left ventricular pressure (Millar) and volume (conductance catheter) were measured in pentobarbital anaesthetised male Göttinger mini-pigs (15-17 kg). Data were acquired at baseline and 10 minutes following intravenously administered SIN-1 (1.0 - 30.0 $\mu g \cdot kg^{-1}$) or saline vehicle. Pressure-volume analysis was then used to provide indices of preload (end-diastolic volume; EDV), afterload (arterial elastance; Ea), contractility (end-systolic elastance; Ees) and stroke volume (SV). This approach allowed quantification of changes in myocardial oxygen requirements by computation of pressure-volume area (PVA), an index of myocardial oxygen consumption (Suga, 1979).

SIN-1 ($\mu g \cdot kg^{-1}$)	EDV (ml)	Ea ($mmHg \cdot Hg^{-1}$)	Ees ($mmHg \cdot Hg^{-1}$)	SV (ml)	PVA ($ml \cdot mmHg$)
baseline	25.4 ± 1.2	10.8 ± 0.8	5.8 ± 0.9	11.3 ± 0.7	2477 ± 207
1.0	24.6 ± 1.4	10.3 ± 0.7*	5.7 ± 0.9	11.4 ± 0.6	2414 ± 262
3.0	23.9 ± 1.4*	9.5 ± 0.7*	5.4 ± 0.8	11.3 ± 0.6	2171 ± 192*
10.0	22.2 ± 1.6**	9.1 ± 0.8*	5.4 ± 0.7	10.6 ± 0.6	1868 ± 232**
30.0	20.0 ± 1.2**	7.0 ± 0.8**	5.4 ± 0.8	10.9 ± 0.8	1357 ± 228**

*Data are presented as mean ± S.E. of 6 animals, with * P<0.05, ** P<0.01 compared to baseline*



The figure shows representative left ventricular pressure-volume loops from one animal at baseline (larger loop) and following the 30 $\mu g \cdot kg^{-1}$ dose of SIN-1. SIN-1 had no significant effect on contractility (Ees) but caused dose-related falls in both preload (EDV) and afterload (Ea) with no net change in stroke volume. Pressure-volume analysis relvealed that the observed decrease in PVA (46 ± 7% following the highest dose) was elicited by the combined effects of reductions in both EDV and Ea. In conclusion, SIN-1 caused a marked and dose-related reduction in myocardial oxygen demand in the anaesthetised mini-pig which was due to a fall in both preload and afterload.

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397P INTERACTIONS BETWEEN PHORBOL-12,13-DIBUTYRATE AND LIGNOCAINE IN SHEEP PURKINJE FIBRES

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Intracellular messengers modulate the actions of many drugs, for example β-adrenoceptor antagonists. However, there is little information about the mechanisms responsible for regulation of other antiarrhythmic drugs, in particular lignocaine, even though it is known to block the inward sodium and calcium currents (Mitchell & Plant, 1988). In the central nervous system phorbol esters, which activate protein kinase C, potentiate the actions of lignocaine (Austin *et al.*, 1991). The present study investigated the interaction between phorbol-12,13-dibutyrate (PDBu) and lignocaine (Lig) on action potential parameters in sheep Purkinje fibres.

Sheep hearts (n=20) were obtained from the abattoir. Free running Purkinje fibres were removed from the left ventricle, pinned to the base of a tissue bath and superfused (3ml.min^{-1}, at 37°C) with oxygenated (95/5%, O_2/CO_2) normal Tyrode's solution. Action potentials were recorded by conventional glass microelectrode techniques. The tissue was electrically stimulated with bipolar electrodes at 1Hz with supra-threshold stimuli (1ms). Following equilibration (>60 min), Lig (5-50 μM) was added alone or with PDBu (200 nM) for 25 min. Other tissues were superfused with either PDBu, 200 nM, or normal Tyrode's solution. Recordings were made at equivalent time points to those with Lig. Action potentials were digitised and stored on computer for analysis. Comparisons were made between groups using a MANOVA (repeated measures) and Duncan's multiple comparison test. Data are presented as mean ± S.E.M.

PDBu increased ($p<0.05$) the hyperpolarization, which was reduced by Lig ($p<0.05$) in a dose dependent manner. Separately PDBu and Lig shortened ($p<0.05$) the action potential duration at 90% (APD_{90}) and 50% repolarization, and this effect was additive when given in combination at concentrations of Lig greater than 10 μM (table).

These results show that the hyperpolarization caused by PDBu is decreased by Lig and that shortening of the action potential by PDBu and Lig is increased when given together. Hence, the effects of Lig may be modified by intracellular modulatory enzymes.

[Lig] or	Hyperpolarization (mV)				APD_{90} (ms)			
Equivalent time	Control	Lig	PDBu	Lig + PDBu	Control	Lig	PDBu	Lig + PDBu
0\0 min	-4.0±0.2	-5.02±0.9	-4.4±0.3	-3.4±0.4	373±17	372±20	321±15	350±32
5μM\25 min	-3.9±0.2	-4.7±0.9	-13.2±1.6*†	-7.3±1.3	370±22	306±13*	301±13*	263±12
20μM\75min	-3.8±0.2	-3.4±0.7	-10.0±1.6*†	-3.9±0.4	348±18	227±11*†	232±14*†	165±14
50μM\100min	-3.7±0.2	-2.26±0.6	-8.9±1.2*†	-1.5±0.3*	344±20	177±8*†	222±14*†	127±11

* $p<0.05$ Drug vs control; † $p<0.05$ Lig or PDBu vs Combination

This work was supported by the British Heart Foundation

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398P MEDIATORS INVOLVED IN THE ANTIARRHYTHMIC EFFECT OF ISCHAEMIC PRECONDITIONING IN GUINEA-PIG ISOLATED HEARTS

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Exposing the heart to a brief period of ischaemia renders it more resistant to injury resulting from subsequent prolonged periods of ischaemia, a phenomenon termed ischaemic preconditioning. The protection includes a reduction in the severity of ventricular arrhythmias and involves the release of endogenous mediators (Liu et al., 1991; Vegh et al. 1992). The aim of the present study was to examine a possible role in preconditioning for these mediators using guinea-pig isolated hearts subjected to global ischaemia.

Hearts were isolated from guinea pigs (weighing 350-450 g) and were perfused with a modified Krebs Henseleit solution at a constant rate of 7-10 ml/min (baseline coronary perfusion pressure of 50-60 mmHg). After stabilisation, hearts were subjected to 30 min of zero flow global ischaemia during which the occurrence of ventricular tachycardia (VT) and ventricular fibrillation (VF) were analysed and compared to hearts which were preconditioned. Preconditioning involved exposure of the hearts to a 4 min period of zero flow global ischaemia followed by 10 min of reperfusion and 30 min of global ischaemia. During global ischaemia hearts were paced at 4.5 Hz and were submerged in an organ bath to keep the temperature constant at 37°C. The role of adenosine, prostanoids and nitric oxide (NO) in the antiarrhythmic effect of ischaemic preconditioning in this model were assessed by the use of a non-selective adenosine receptor antagonist (8-(p-sulfonyl)theophylline; 8-SPT), and inhibitors of cyclooxygenase (indomethacin; IND) and nitric oxide synthase (N^G-nitro-l-arginine; NO_2ARG).

Global ischaemia for 30 min induced VT and VF in 85.7% and 35.7% of 14 hearts respectively. The onset times were15.8±0.8 and 15.4 ±1.1 min respectively. In another 14 hearts which were preconditioned the incidences of VT and VF during 30 min of global ischaemia were markedly reduced (14% and 0%, respectively; $P<0.01$). The incidences of VT and VF in 8-SPT (50 μM) pretreated and preconditioned hearts were 100% ($P<0.05$) and 0% (ns), in IND (5 μM) pretreated and preconditioned hearts, 71% ($P<0.05$) and 0% (ns) and in NO_2 Arg (100 μM) pretreated hearts 33% and 11% (ns) respectively. No pretreatment modified the antifibrillatory effect of preconditioning. It is thus possible that, in this model, adenosine, prostanoids (prostacyclin?) and NO contribute to the marked reduction in VT that occurs following this form of preconditioning by global ischaemia. The possible interactions of these mediators, as well as their mechanisms of action, remain to be elucidated.

Stefan Chlopicki and Maciej Slawinski were guest research fellows from the Department of Pharmacology, Copernicus University, Cracow, Poland and were supported by 'Go-West' grants from the European Economic Commission.

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399P BLOCKING ADENOSINE RECEPTORS FAILS TO MODIFY THE PRONOUNCED ANTIARRHYTHMIC EFFECT OF ISCHAEMIC PRECONDITIONING IN THE CANINE MYOCARDIUM

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Short periods of myocardial ischaemia, induced either by complete coronary artery occlusion or rapid ventricular pacing, markedly reduce the severity of ischaemic damage (infarct size, Murry *et al.*, 1986) and the life-threatening ventricular arrhythmias (Vegh *et al.*, 1990) that result from a subsequent and more severe period of ischaemia. This is termed ischaemic preconditioning (PC). The importance of the phenomenon is that, if the mechansisms were known, they could almost certainly be exploited pharmacologically (Parratt 1993). Adenosine has been suggested as the mediator in reducing infarct size but its possible role in the antiarrhythmic effects of preconditioning has not as yet been explored in large animal models. We examined this possibility in chloralose-urethane anaesthetised dogs preconditioned (Vegh *et al.*, 1990; 1992) by two 5 min occlusions of the anterior descending branch of the left coronary artery (LAD) followed, 20 min later, by a 25 min occlusion of that same artery. The adenosine antagonist 8-sulphophenyltheophylline (8-SPT) was given in a dose (1 mg kg^{-1}, which prevented the coronary vasodilator effect of locally infused adenosine up to 10 μg kg^{-1}) 10 min before each occlusion into a side branch of the LAD.

Although 8-SPT increased the number of ventricular beats (VPBs) during the first preconditioning occlusion (from 9 ± 3 to 61 ± 20; $P < 0.01$ and the incidence of ventricular fibrillation (VF) during this initial occlusion from 4/20 to 5/11; $P < 0.05$, Mann Whitney U test). It failed to modify the antiarrhythmic effects of preconditioning during the prolonged (25 min) occlusion (VPBs 72 ± 21 in PC dogs and 127 ± 53 in PC dogs given 8-SPT compared with 497 ± 109 in the controls ($P < 0.01$). VF was 0%, 0% (ns) and 47% ($P < 0.05$) respectively in these three groups and the number of episodes of ventricular tachycardia (VT) per dog were 0.7 ± 0.4, 1.0 ± 0.7 (ns) and 5.9 ± 1.7 respectively ($P < 0.01$).

These results imply that adenosine, although an endogenously released antiarrhythmic substance, does not mediate the antiarrhythmic effects of preconditioning in this model; other mediators such as nitric oxide, bradykinin and prostanoids (Vegh *et al.*, 1990; 92) are involved.

This work was supported by the Wellcome Trust, the British Council (in collaboration with the Hungarian National Committee for Technical Development) and the Hungarian State Government (OTKA).

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400P *IN VITRO* PHARMACOLOGICAL PROFILE OF SELECTED ADENOSINE A_2 ANTAGONISTS

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We characterized the "in vitro" pharmacological profile of the putative A2 adenosine receptor antagonists, the xanthine derivative (E)7-methyl-8- (3,4-dimethoxystyryl-1,3-dipropylxanthine (KF 17837) (Shimada et al., 1992), and two non-xanthine compounds, the 5-amino-9-chloro-2-(2-furyl)1,2,4-triazolo-[1,5-c] quinazoline (CGS 15943) (Francis et al., 1988) and the 5-amino-8-(4-fluorobenzyl)-2-(2-furyl)-pyrazolo[4,3-e]-1,2,4-triazolo- [1,5-c]pyrimidine (8FB-PTP) (Gatta et al., 1993). Binding affinity at A1 and A2 receptors was tested on bovine brain cortex and striatal membranes using [3H]CHA and [3H]CGS 21680, respectively. Antagonism to A2-mediated effects was tested in bovine coronary artery relaxation (Conti et al., 1993) and antiaggregatory activity in rabbit platelets (Gatta et al., 1993) induced by 5'-N-ethylcarboxamidoadenosine (NECA). Antagonism to the negative chronotropic A1-mediated responses induced by 2-chloro-N6-cyclopentyladenosine (CCPA) was tested in rat atria (Conti et al., 1993). Results are reported in the following Table:

ADENOSINE ANTAGONIST	BINDING STUDIES		RABBIT PLATELETS		BOVINE CORONARY ARTERY		RAT ATRIA	
	A1 Ki (nM)	A2 Ki (nM)	pA2	Schild-plot slope	pA2	Schild-plot slope	pA2	Schild-plot slope
8FB-PTP	2.04 (1.77-2.34)	0.074 (0.064-0.086)	8.20 (7.69-8.69)	0.97 (0.90-1.03)	7.98 (6.92-9.03)	1.08 (0.91-1.24)	8.71 (8.07-9.35)	0.96 (0.88-1.04)
CGS 15943	2.40 (2.07-2.78)	0.95 (0.79-1.14)	7.43 (6.66-8.19)	1.04 (0.92-1.16)	n.m.(1 μM)	-	9.62 (8.72-10.5)	1.00 (0.89-1.11)
KF 17837	273 (164-343)	73.6 (60.8-89.1)	n.m.(1 μM)	-	n.m.(1 μM)	-	n.m.(1 μM)	-

n.m.: not measurable. 95% confidence limits or highest concentrations tested are in parenthesis.

The results indicate that the new non-xanthine A2 antagonist 8FB-PTP is the most potent compound on A2 bovine striatal receptors and shows clear competitive antagonistic properties in reliable functional assays. Conversely, the xanthine derivative KF 17837, which was presented as selective A2 antagonist in binding studies, has been found to lack A2 antagonistic properties in "in vitro" functional models. From the present data, there is the basis to progress with further synthetic efforts in order to improve A2 receptor selectivity of non-xanthine derivatives.

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401P EFFECT OF H7 ON ADENOSINE A_2 RECEPTOR DESENSITIZATION IN NG108-15 CELLS

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Pretreatment of NG108-15 cells with either forskolin or the adenosine A_2 receptor agonist, N-ethylcarboxamido adenosine (NECA), produces a decrease in the subsequent ability of NECA to stimulate adenylyl cyclase activity in homogenates of these cells (Keen et al., 1989; 1992). The desensitization produced by forskolin can be abolished by the protein kinase inhibitor H7 (Krane and Keen, 1991), suggesting that the effect of forskolin is mediated via activation of protein kinase A (PKA). In this study we have investigated the effect of H7 on desensitization produced by pretreatment with NECA.

Confluent NG108-15 cells (passage 19-26) were incubated at 37°C for 2-17h in Dulbecco's modified Eagle's medium containing either 10μM NECA or vehicle. In each case, parallel incubations were carried out in the absence or presence of 50μM H7. At the end of the incubation period, cells were harvested and washed three times in phosphate buffered saline and then frozen at -80°C until required. Adenylyl cyclase assays were carried out on homogenates of these cells as previously described (Keen et al., 1989). Data were compared using paired *t*-tests and $P<0.05$ was considered significant.

Pretreatment with NECA and/or H7 for 17h appeared to produce a decrease in basal adenylyl cyclase activity, but this effect was not significant. In homogenates of control cells, basal activity was 32.6±21.6 pmol cAMP.min^{-1}.mg protein^{-1}. In homogenates of cells pretreated with NECA, H7 or NECA and H7, respectively, activities were 16.8±8.2, 22.7±8.7 and 14.2±7.8 pmol cAMP.min^{-1}.mg protein^{-1} (all values mean±s.e.mean, n=3). Pretreatment with NECA for 17h produced a marked and significant reduction in the subsequent response to 100μM NECA, which was unaffected by the presence of H7. In control cell homogenates, 100μM NECA produced an increase in adenylyl cyclase activity of 116.0±21.9% above basal. In homogenates of cells pretreated with NECA, H7 or NECA and H7, respectively, the increases in adenylyl cyclase activity produced by 100μM NECA were 6.3±5.4%, 106.3±18.4% and 7.3±2.7% (all values mean±s.e.mean, n=3).

The decrease in the subsequent response to NECA produced by the NECA pretreatment was a time-dependent process, which could be well described by a single exponential. The rate of desensitization was unaffected by H7, the rate constant obtained in the presence of NECA alone being 0.566±0.090h^{-1} and that obtained in the presence of NECA and H7 being 0.558±0.066h^{-1} (all values mean±s.e.mean, n=3).

Thus H7 affected neither the extent nor the rate of desensitization produced by pretreatment of NG108-15 cells with NECA. This suggests that PKA is not involved in agonist-mediated desensitization of the adenosine A_2 receptor in these cells.

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402P SPECIES-DEPENDENT CARDIOVASCULAR EFFECTS OF SDZ WAG 994, A SELECTIVE ADENOSINE A_1 RECEPTOR AGONIST

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The pharmacological effects of adenosine are mediated via P_1 purinoceptors of which four subtypes, designated A_1, A_{2A}, A_{2B} and A_3, have now been cloned (Abbracchio et al., 1993). We here describe the pharmacological properties of 6-cyclohexyl-2'-O-methyladenosine (SDZ WAG 994) a potent, selective and orally active adenosine A_1 receptor agonist.

Radioligand binding to the A_1 and A_{2A} receptors of pig striatum (see Fozard & Milavec-Krizman, 1993) and the effects on activated adenylate cyclase of rat adipocyte membranes (Schoeffter & Hoyer, 1988), rat spleen (Fozard & Milavec-Krizman, 1993) and precontracted guinea-pig aorta (Martin, 1992) were measured by established methods. Cardiovascular effects were measured in spontaneously hypertensive (SH) rats, either by means of an implanted aortic cannula or the Data Science telemetry system, in conscious dogs via a punctured carotid artery exteriorized in a loop of skin and in salt-depleted Squirrel monkeys by the tail-cuff method in animals trained to sit quietly in restraining chairs. Blood samples were withdrawn for measurements of plasma renin activity (PRA).

SDZ WAG 994 is a potent (K_D 23 ± 2 nM, n=5) and selective (1090-fold vs A_{2A} receptors) displacer of binding to pig striatal A_1 receptors and behaves a full agonist at the A_1 receptor coupled negatively to adenylate cyclase in rat adipocytes (pEC_{50} 6.43 ± 0.17, n=3) and which induces contraction of rat spleen (pEC_{50} 7.06 ± 0.06, n=13). The compound has negligible affinity (pIC_{50} < 4, n=5) for the A_{2B} receptor which mediates relaxation of guinea-pig aorta. In SH rats SDZ WAG 994, 0.05 - 0.5 mg/kg p.o., caused dose-related and sustained (> 5 h) falls in blood pressure and heart rate and suppressed PRA; the cardiovascular effects could be immediately and fully reversed by an intravenous injection of 8-(p-sulphophenyl)theophylline, 20 mg/kg. SDZ WAG 994 given via the food at 0.4 and 1.2 mg/kg/day for 7 or 14 days, respectively, induced dose-related hypotension and bradycardia which were well maintained throughout the treatment periods. In dogs, SDZ WAG 994, 1-3 mg/kg p.o. induced hypotension associated with tachycardia; the latter was abolished in animals treated with chlorisondamine 0.25 mg/kg i.v. In salt-depleted Squirrel monkeys, SDZ WAG 994, 0.2 - 0.6 mg/kg p.o. caused dose-related and sustained (> 5 h) bradycardia and suppression of PRA but no change in blood pressure.

Thus, SDZ WAG 994, characterized in vitro, as a selective A_1 receptor agonist, induces markedly different cardiovascular responses in rats, dogs and Squirrel monkeys. The basis for these differences must remain speculative but may reflect variations in the distribution and/or function of A_1 receptors in the different species.

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403P INVOLVEMENT OF DIFFERENT CALCIUM SOURCES IN THE INHIBITION OF PHENYLEPHRINE CONTRACTIONS OF GUINEA-PIG AORTA BY NECA

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The importance of different calcium sources in the vasodilator response of 5'-N-ethylcarboxamidoadenosine (NECA) was studied in phenylephrine (PE) contracted guinea-pig thoracic aorta. In this preparation vasodilatation is thought to be mediated by the A_{2B}-adenosine receptor subtype (Martin 1992).

Two 5mm rings of endothelium-denuded guinea-pig thoracic aorta, from a single guinea-pig, were mounted, under a tension of 1g, in 50ml tissue baths containing Krebs bicarbonate solution with 2.52mM calcium ('calcium' preparations). Two more 5mm rings of endothelium-denuded guinea-pig thoracic aorta from the same guinea-pig were mounted in 50ml tissue baths containing Krebs bicarbonate solution where calcium was omitted and 4mM EDTA added ('calcium-free' preparations).

A contraction to a sub-maximal dose ($3x10^{-6}$M) of PE was obtained in all preparations, which were then washed and incubated with 'normal' Krebs bicarbonate solution for 15 minutes. The 'calcium-free' preparations were then washed in 'calcium-free' Krebs solution. NECA (10^{-5}M) or ryanodine (10^{-5}M) was added to one of the normal and 'calcium-free' preparations. After 10 minutes a final contraction was obtained to $3x10^{-6}$M PE. The results are reported as (2^{nd} contraction/ 1^{st} contraction)x100 ± sem in 'test' (in the presence of the test substance) and 'control' (in the absence of the test substance). Statistical significance ($P<0.05$) was tested using Student's t-tests of paired data.

10^{-5}M NECA caused a significant reduction in PE contractions in both 'normal' (79.7±3.9%(n=9)) and 'calcium-free' (55.2±9.1%(n=9)) compared with their corresponding controls (133.1±9.8% (n=9) and 87±14.9%(n=9) respectively). 10^{-5}M of the intracellular calcium-release blocker ryanodine significantly reduced the test PE contractions in both 'normal' (100.1±3.6%(n=5)) and 'calcium-free' (28.3±11.6%(n=6)) compared with their controls (117.1±3.8%(n=5) and 106.8±19.7%(n=6) respectively).

Further experiments were conducted using 'normal' Krebs bicarbonate solution with 10^{-5}M ryanodine present at all times. In this situation 10^{-6}M NECA was still found to significantly reduce the PE contractions (77.3±12.4% (n=5)) compared with controls (111.4±9.3% (n=5)).
NECA had a similar action to ryanodine in 'calcium-free' preparations indicating that A_{2B}-adenosine receptors can block contractions dependent on an intracellular source of calcium. The inhibition by NECA of PE contractions in the presence of calcium and 10^{-5}M ryanodine indicates that A_{2B}-adenosine receptors are also capable of inhibiting contractions dependent on an extracellular source of calcium. In summary, NECA, via A_{2B}-adenosine receptor stimulation, is able to inhibit PE contractions, in guinea-pig thoracic aorta, mediated by intracellular and extracellular calcium

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404P NOT ALL ADENOSINE RECEPTOR BLOCKING DRUGS AUGMENT ADENOSINE A_1-RECEPTOR-MEDIATED CONTRACTIONS IN RAT COLON

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We have previously reported that 8-phenyltheophylline (8-PT) not only produced rightward shifts of the concentration-effect curves to adenosine A_1-receptor agonists, but also augmented the maximum contraction in rat isolated colonic mucularis mucosae (RCMM; Reeves et al., 1993). We have now further investigated the effects of several adenosine receptor antagonists on responses to the non-selective adenosine agonist 5′-N-ethylcarboxamidoadenosine (NECA) in this preparation.

Contractions to NECA, and their modification by receptor blocking drugs, were studied using methods described previously (Reeves et al., 1993). As previously, 8-PT (0.1-3μM) both produced rightward shifts of concentration-effect curves [apparent mean pK_B (95% confidence limits) =6.6 (6.4-6.8), n=20], and augmented maximum contractions (by 83.3±15.6% at 1μM 8-PT, n=4, $p< 0.01$) to NECA in RCMM. Indomethacin (3μM) inhibited NECA-induced contractions and reduced the maximum response by 35.4±5.8% (n=8, $P<0.05$), whilst a higher concentration (10μM) produced no further inhibition. In the presence of indomethacin (3μM), 8-PT (1μM; n=10) still caused a 54.7±6.8% increase ($p<0.05$) in the maximum response to NECA.

8-p-sulphophenyltheophylline (10μM) and caffeine (100μM) showed similar profiles to 8-PT, with apparent pK_B values of 5.9 and 4.3 for inhibition of the contractile response, and increases in the NECA maxima of 37.2±7.3% (n=5, $p<0.05$) and 45.3±5.4% (n=3, $p<0.05$), respectively. Interestingly, a low concentration of isobutylmethylxanthine (1μM) increased the maximum contractile response to NECA in RCMM by 49.8±6.2% (n=6, $p<0.05$) without producing a rightward shift of the NECA curve, whereas a higher concentration (10μM) virtually abolished basal tone. In contrast, the non-xanthine antagonist, CGS15943A (Ghai et al., 1987) at 0.03 and 0.1μM, produced rightward shifts of NECA concentration-effect curves [mean apparent pK_B=8.3 (7.9-8.8), n=7] without increasing the maximum contractile response to NECA. At the concentrations tested, none of the antagonists had an effect on contractions to carbachol.

In conclusion, the present data suggest that adenosine A_1 receptor-induced contractions in RCMM are mediated, in part, via cyclooxygenase products (e.g. prostanoids). The mechanism(s) of the observed augmentation of contraction in RCMM is unknown, but is likely to be, at least in part, independent of cyclooxygenase. To date, augmentation of the contractile response to NECA in the RCMM has been observed only for methylxanthine-containing antagonists.

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405P IONIC DEPENDENCE OF DEPOLARISING RESPONSES EVOKED BY α,β-METHYLENE ATP IN THE RAT ISOLATED VAGUS NERVE

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In general, the ionic selectivity and permeability of ion channels gated by adenosine 5'-triphosphate (ATP) is similar to that reported for 5-HT_3 receptor-gated cation channels (see Bean, 1992 and Yakel, 1992). Recently, we reported that the rat isolated vagus nerve is depolarised by ATP and the stable analogue of ATP, α,β-methylene ATP (Trezise *et al.*, 1993). In an attempt to determine whether these responses are mediated via the opening of receptor-gated cation channels we have compared the ionic dependence of responses to α,β-methylene ATP with that of responses to 5-HT, which are mediated via activation of 5-HT_3 receptors in this preparation (Ireland & Tyers, 1987).

Segments of cervical vagus nerve were removed from male AHA rats (200-300g), desheathed and placed in a chamber for the recording of extracellular d.c. potentials using the 'grease-gap' technique, as previously described (Trezise *et al.*, 1993). Concentration-effect curves to either α,β-methylene ATP or 5-HT were constructed by addition of sequentially increasing doses of drug to the perfusate for periods of 2min at 45min intervals. Data were calculated as a percentage of the response to a maximally effective concentration of 5-HT (10μM) administered at the start of the experiment. In control preparations superfused with normal Krebs solution (Trezise *et al.*, 1993) the maximal amplitude of depolarisation to α,β-methylene ATP (0.3-300μM) and 5-HT (0.1-30μM) amounted to 206 ± 11% (mean ± s.e.mean, n=29) and 106 ± 7% (n=25), respectively. Equimolar substitution of Na^+ with either Tris or glucosamine significantly ($P<0.05$) reduced responses to α,β-methylene ATP to 104 ± 7% and 82 ± 7%, respectively and those to 5-HT to 37 ± 5% and 17 ± 7%, respectively. Replacement of NaCl with Na isethionate had no significant effect on responses to α,β-methylene ATP (194 ± 27%) but produced a small, significant ($P<0.05$) increase in responses to 5-HT (120 ± 10%). Removal of both Ca^{2+} and Mg^{2+} (+ 1mM EDTA) markedly augmented depolarisations to both 5-HT (204 ± 25%) and α,β-methylene ATP (707 ± 127%), whilst elevating extracellular Ca^{2+} from 1.3mM to 7.8mM inhibited these responses (α,β-methylene ATP; 113 ± 15%; 5-HT; 64 ± 7%). Addition of tetrodotoxin (0.3μM) or nifedipine (1μM), or removal of Mg^{2+} or Ca^{2+} alone, had no significant effects on responses to either agonist.

These data demonstrate a marked similarity between the ionic dependence of depolarisations produced by α,β-methylene ATP and 5-HT in the rat isolated vagus nerve. These findings provide corroborative evidence that membrane currents evoked by α,β-methylene ATP, as is the case with 5-HT, are carried by cation channels that allow the passage of Na^+, and to a lesser degree, larger positively charged species such as Tris and glucosamine. Tetrodotoxin-sensitive Na^+ channels and L-type voltage-operated Ca^{2+} channels are not involved. The attenuation of responses to α,β-methylene ATP by divalent cations is consistent with the hypothesis that these ions interfere with the permeation of Na^+ through these channels. However, other possible explanations for this action such as an effect on receptor desensitisation cannot, at this stage, be excluded.

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406P RELATIVE AGONIST POTENCIES AT P_2 PURINOCEPTORS IN RAT AND GUINEA-PIG VAS DEFERENS

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The classification of P_2 purinoceptors depends largely upon comparison of agonist potency rank orders (Kennedy, 1990), and therefore accurate quantification of agonist potencies is important. In this study we have attempted to quantify the relative potencies of a range of purine nucleotides in causing contraction of isolated vas deferens of the rat and guinea-pig.

Vasa deferentia removed from rats and guinea-pigs were bisected transversely to obtain two epididymal and two prostatic preparations from each animal. The preparations were mounted under 0.5g resting tension for 30 min in gassed modified Krebs containing indomethacin (5.8μM), and a concentration-effect (C-E) curve was constructed to α,β-meATP (10nM-0.1 mM), using sequential dosing. Following completion of the first C-E curve, preparations were left unchallenged for one hour. Second C-E curves to agonists were then constructed, one preparation always received α,β-meATP, thus serving as a control, the other three preparations each received different test agonists (10nM-0.1mM, except ATP 10nM-1mM). Contractile responses were expressed as arithmetic mean percentage (± S.E.M) of the response to 100mM KCl. Equi-effective molar ratio (EMR) values were calculated for agonists at the 50% response level for the first C-E curve to α,β-meATP, and are shown as geometric means (95% confidence limits) from 4 experiments.

In the present study little or no difference in sensitivity to α,β-meATP was seen between epididymal and prostatic preparations in rat or guinea-pig vasa deferentia. This is in contrast to a previous report (Sneddon & Machaly, 1992). Although agonist concentration-effect curves did not always achieve a well defined maximum, curves for β,γ-meATP, β,γ-imidoATP and ATPγS appeared to be parallel to that for α,β-meATP in both species, and therefore EMR values were calculated (see table). On rat vas deferens, both 2MeS-ATP and ATP produced only small contractions at high concentrations, and were at least 100 times weaker than α,β-meATP. On guinea-pig vas deferens, the threshold concentrations for ATP (100nM) and 2MeS-ATP (300nM) were only slightly higher than that for α,β-meATP (30-100nM). However, the concentration-effect curves were much shallower than that for α,β-meATP. At the highest concentrations tested ATP (1mM) and 2MeS-ATP (0.1mM) produced contractions (%100mM KCl) of 48±9.8 and 35.4±4.4. ADP, AMP and adenosine produced little or no contraction in either species in concentrations up to 0.1mM.

	Species	α,β-meATP	β,γ-imidoATP	ATPγS	β,γ-meATP
EMR (n=4)	**Rat**	1	4 (2-10)	11 (9-13)	18 (12-26)
(95% C.L)	**Guinea-pig**	1	10 (3-29)	4.7 (1-18)	9 (3-25)
***MAXIMUM**	**Rat**	79±11	81±22	59±16	56±13
(± S.E.M)	**Guinea-pig**	46±4	61±6	72±4	64±4

*Contractile response at the highest concentration (0.1mM) tested.

Overall, the results with α,β-meATP, β,γ-meATP and β,γ-imidoATP are consistent with the conclusion that contraction of rat and guinea-pig vas deferens is mediated by P_{2X} purinoceptors (Kennedy, 1990), and these results provide a useful profile for comparison with other tissues containing purinoceptors. Although it was not possible to obtain EMR values for ATP and 2MeS-ATP, both compounds were clearly less potent than α,β-meATP and more potent in the guinea-pig than in the rat. Both of these compounds are subject to rapid metabolism, which could influence their potency. Another possibility is that rat and guinea-pig vas deferens contain, in addition to P_{2X} purinoceptors, different proportions of other types of purinoceptor, mediating either contraction or relaxation. Further work will be necessary to investigate these possibilities.

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407P BOTH P_{2Y} PURINOCEPTORS AND NUCLEOTIDE RECEPTORS STIMULATE ARACHIDONIC ACID RELEASE FROM CULTURED AORTIC ENDOTHELIAL CELLS

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Extracellular ATP regulates bovine aortic endothelial cell phospholipase C by interacting with both P_{2Y} purinoceptors and nucleotide receptors (Wilkinson et al, 1993); these two receptors produce differential responses with respect to both second messengers and smooth muscle relaxation, with only the P_{2Y} induced relaxation being sensitive to the cyclooxygenase inhibitor indomethacin (Wilkinson et al, 1994). Previous studies have shown that ATP can stimulate the production of prostacyclin by acting on endothelial P_{2Y} purinoceptors (Carter et al, 1988). Here we ask whether the two receptors for ATP found on endothelial cells are differentially linked to production of the prostanoid precursor arachidonic acid.

Bovine aortic endothelial cells were grown to confluence in 24 well plates and labelled for 18 h with 0.25 μCi/0.5ml/well of [^{3}H]arachidonic acid. The cells were washed 4 times in 500 μL of balanced salt solution with 1.8 mM Ca^{2+} and then stimulated with agonists. The supernatant was removed and counted. The identity of [^{3}H]arachidonic acid in cell extracts was confirmed by thin layer chromatography. Data are mean ± s.e.mean, dpm x 10^{-3}, n = 3.

On stimulation with 300 μM ATP (an agonist at both receptor types) there was an increase above the control level at 30 s reaching a maximum at 5 min (3-4 fold over the control level at this time), after which the level of [^{3}H]arachidonic acid in the medium remained level. Essentially the same result was seen when stimulation was with 300μM UTP (an agonist at nucleotide receptors but not P_{2Y} purinoceptors) and with 30 μM 2-methylthio ATP (2MeSATP: an agonist at P_{2Y} purinoceptors but not nucleotide receptors). In each case the rate of rise to a maximum at 5 min was essentially identical, reaching the same final level (e.g. control, 4.91 ± 0.41; ATP, 17.6 ± 0.34; 2MeSATP, 16.5 ± 0.34; UTP; 16.6 ± 0.75). Concentration response curves constructed with 2 min incubation gave an EC_{50} for ATP and UTP of about 10 μM and for 2MeSATP of about 1 μM. In the presence of low Ca^{2+} (buffered to 80-120 nM) most of the response was lost in each case.

The results show that in cultured aortic endothelial cells the production of arachidonic acid can be modulated by both P_{2Y} purinoceptors and nucleotide receptors. This suggests that both receptor types could be involved in the control of prostacyclin production.

This work was supported by The Wellcome Trust.

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408P α,β-METHYLENE ATP BUT NOT URIDINE-5'-TRIPHOSPHATE-EVOKED DEPOLARISATIONS OF THE RAT SUPERIOR CERVICAL GANGLION ARE ANTAGONISED BY A P_{2X}-PURINOCEPTOR ANTAGONIST

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In the cardiovascular system there is some evidence in favour of the presence of extracellular receptors that are activated specifically by uridine-5'triphosphate (UTP) rather than by adenosine 5'-triphosphate (ATP), i.e., pyrimidinoceptors, however it is not known if these receptors exist within the nervous system. We have recently reported that the rat superior cervical ganglion (SCG) depolarises in response to both ATP and UTP, the former effect being mediated by the activation of P_{2X}-purinoceptors and the latter via a suramin-insensitive mechansim (Connolly et al., 1993). Our present aim was to examine further the possibility that the rat SCG contains pyrimidinoceptors by studying the effect of an isomer of PPADS (a novel putative P_{2X}-purinoceptor antagonist (Lambrecht *et al.*, 1992)), i.e. pyridoxal-phosphate-6-azophenyl-2',5'-disulphonic acid (IsoPPADS) on responses evoked by alpha-beta-methylene-ATP (αβMeATP) and UTP. Using methods described before (Connolly *et al.*, 1993), ganglia were placed in a chamber and perfused with physiological salt solution at 25±1°C, pH 7.4, equilibrated with 5% CO_2/95%O_2, containing 2mM potasium (K^+) and 0.1mM calcium. The d.c. potential between the ganglion and the postganglionic internal carotid nerve was recorded across a greased barrier. Adenosine, αβMeATP or UTP were added to the perfusate for 2 min and muscarine or K^+ for 1 min with a minimum 20 min between applications. Responses of 7 ganglia in the absence (control) and presence of IsoPPADS (≥ 30 min incubation) were compared for Statistical significance using a paired Student's t test; ** = P <0.01.

Table 1. Effect of IsoPPADS on the response (μV, mean ± s.e.mean) to depolarising agents and adenosine.

Treatment\Agent	αβMeATP (100μM)	UTP (100μM)	Adenosine (100μM)	Muscarine (100nM)	K^+ (5mM)
CONTROL	132 ± 12	161 ± 32	-114 ± 9	234 ± 27	154 ± 12
IsoPPADS (10μM)	73 ± 11**	175 ± 25	-123 ± 24	154 ± 36**	151 ± 28

Depolarisations mediated by the P_{2X}-purinoceptor agonist αβMeATP were significantly reduced, whereas depolarisations caused by UTP or K^+ and hyperpolarisations evoked by adenosine were unaltered in the presence of IsoPPADS (Table 1). The mechanism responsible for antagonism by IsoPPADS of muscarine evoked depolarisation is unknown. Our results are consistent with the proposal that like PPADS, IsoPPADS is an antagonist of P_{2X}-purinoceptors and that the UTP-evoked depolarisation of the rat SCG is not mediated via P_{2X}-purinoceptors, rather it is mediated by activation of pyrimidinoceptors.

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409P MODULATION BY SIN-1 OF THE RESPONSES TO ATP AND TO NORADRENALINE IN RAT ISOLATED MESENTERIC RESISTANCE ARTERIES

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The mechanism by which nitric oxide (NO) modulates contraction induced by adenosine triphosphate (ATP) and noradrenaline (NA), which are known to act through different mechanisms, was investigated in small mesenteric artery of the rat using a NO donor, 3-morpholino sydnonimine-N-ethylcarbamide (SIN-1). In this resistance artery, the contraction induced by ATP is associated with depolarization and release of calcium from the internal source. NA, in addition to its effect on calcium handling, is known to enhance the sensitivity of the contractile proteins to Ca^{2+} ions. Branch II or III of superior mesenteric arteries were dissected from 12-14 week old male Wistar rats. Arterial segments 1.5-2 mm were removed and mounted on a myograph filled with physiological salt solution (composition in mM: NaCl 119, KCl 4.7, KH_2PO_4 0.4, $NaHCO_3$ 14.9, $MgSO_4$ 1.17, $CaCl_2$ 2.5, glucose 5.5) continuously kept at 37°C and gassed with a mixture of 95% O_2, 5% CO_2 (pH 7.4). Changes in contraction and intracellular Ca^{2+} ions ($[Ca^{2+}]_i$) were determined simultaneously by measuring the fluorescence of trapped fura-2 excited with a dual wave length fluorimeter. The sensitivity of the contractile proteins to calcium was studied on vessels permeabilized with alpha-toxin from *Staphylococcus aureus*. All the experiments were carried out on vessels with intact endothelium. SIN-1 was used at a concentration of 10 μM at which it produced 80% relaxation of vessels pre-contracted with NA (3 μM). Application of calcium (0.1 to 30 μM) induced a concentration-dependent contraction of the vessels permeabilized with alpha-toxin (400 haemolytic units/ml). The calcium sensitivity of the preparations was not affected by SIN-1 (10 μM). SIN-1 decreased both the rise in $[Ca^{2+}]_i$ and contraction induced by ATP (3 mM) and by low concentrations of NA (i.e. less than 3 μM). SIN-1 shifted the concentration-response curves to ATP 4 fold to the right ($P<0.001$) and decreased the maximal response of the agonist from 0.78 ± 0.06 to 0.47 ± 0.1 g ($n = 12$, $P<0.001$). By contrast, SIN-1 only produced a rightward shift of the concentration-response curves to NA by two fold ($n = 12$, $P<0.001$) without affecting its maximal response. In the presence of a maximally active concentration of a calcium entry blocker, nitrendipine (1 μM), SIN-1 depressed the ATP-induced contraction from 0.45 ± 0.07 to 0.18 ± 0.03 g ($n = 21$, $P<0.001$) but did not affect the NA-induced contraction. In conclusion, these results show that, in rat small mesenteric arteries, SIN-1 does not alter the sensitivity of the contractile proteins to Ca^{2+} ions but decreases contractile responses by lowering $[Ca^{2+}]_i$. They also indicate that the contractile response may be differentially affected by SIN-1 depending on the mechanism(s) by which the agonists trigger the elevation of $[Ca^{2+}]_i$.

410P SENSITIVITY OF LIVER AND KIDNEY CATECHOL-O-METHYLTRANSFERASE TO INHIBITION BY TOLCAPONE IN DEVELOPING RATS

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In the majority of peripheral tissues methylation by catechol-O-methyltransferase (COMT) has been suggested to predominate over deamination by monoamine oxidase in the metabolic degradation of catecholamines (Trendelenburg, 1988). This contrast with that found to occur in rat renal tissues, where deamination of catecholamines is far more intense than methylation (Fernandes & Soares-da-Silva, 1993). The liver is endowed with one of the highest COMT activities in the body playing a major role in the coversion of catecholamine neurotransmitters, catechol steroids and xenobiotic catechols into their corresponding inactive methylated derivatives. The present work has studied COMT activities in the liver and kidney of developing rats and evaluated the enzyme sensitivity to inhibition by tolcapone (Ro 40-7592), a new, selective and non toxic inhibitor of COMT for which new therapeutic opportunities are thought to be possible. COMT activity, evaluated by the ability to methylate epinephrine into metanephrine, was determined in liver and kidney homogenates prepared in 0.5 mM phosphate buffer (pH=7.8) of 30, 60 and 90 days old rats (n=4) . The homogenates were incubated for 30 min at 37° C with increasing concentrations of epinephrine (5-500 μM) in the absence of oxygen; the incubation medium contained pargyline (0.1 mM), $MgCl_2$ (0.1 mM), EGTA (1 mM) and S-adenosyl methionine (0.1 mM). Some experiments were performed in the presence of increasing concentrations (0.1, 0.5 and 1.0 μM) of tolcapone. The kinetics (V_{max} in pmol mg protein^{-1} h^{-1}; K_m in μM) of liver COMT were as follows: 30 days old, $V_{max}=3173\pm256$ and $K_m=18\pm1$; 60 days old, $V_{max}=3049\pm423$ and $K_m=19\pm4$; 90 days old, $V_{max}=2436\pm141$ and $K_m=9\pm1$. The V_{max} and K_m values for COMT in renal tissues were not significantly different from those in the liver (30 days old, $V_{max}=3353\pm118$ and $K_m=20\pm2$; 60 days old, $V_{max}=3450\pm285$ and $K_m=24\pm2$; 90 days old, $V_{max}=4368\pm125$ and $K_m=24\pm1$), using the Tuckey-Kramer method. In both the liver and kidney homogenates, tolcapone was found to produce a concentration-dependent decrease of V_{max} values for COMT activity, this being compatible with non-competitive type of inhibition. The IC_{50} (in μM; 95% confidence limits) values for inhibition of liver COMT by tolcapone were as follows: 30 days old, 0.47 (0.46, 0.49); 60 days old, 0.75 (0.42, 1.36); 90 days old, 0.87 (0.75, 1.01). In renal tissues, the IC_{50} (in μM; 95% confidence limits) values for inhibition by tolcapone were 4-fold lower than in liver homogenates, but showed a similar trend to increase with age [30 days old, 0.10 (0.09, 0.10); 60 days old, 0.18 (0.14, 0.24); 90 days old, 0.19 (0.16, 0.22)]. In conclusion, despite similar V_{max} and K_m values for liver and kidney COMT activities in all groups, aging is accompanied by a decreased sensitivity of the enzyme to inhibition by tolcapone. It is also shown that the form of the enzyme in renal tissues reveals a 4-fold increased sensivity to the COMT inhibitior.

This study was supported by grant number PBIC/C/CEN 1139/92 from the JNICT.

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411P THE ACTION OF ENANTIOMERS OF AMLODIPINE ON VOLTAGE-OPERATED CALCIUM CHANNELS IN VASCULAR SMOOTH MUSCLE CELLS

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Amlodipine is a 1,4 dihydropyridine calcium channel antagonist with a long duration of action *in vivo* (Burges et al., 1985), and *in vitro* (Hughes et al.,1991). Therapeutic Amlodipine is a racemic mixture of the enantiomers R(+) and S(-) of amlodipine maleate. We have studied the effects of these enantiomers seperately and in combination on voltage operated calcium channel currents in single vascular smooth muscle cells.

Single vascular smooth muscle cells were isolated enzymatically using collagenase (0.75mg/ml) and papain (0.25mg/ml) from rabbit ear arteries as previously described (Benham & Bolton 1986). Calcium channel currents were measured by the whole cell mode of the voltage clamp technique using Ba^{2+} (110mM) as the charge carrier (Hamill et al., 1981). Concentration response data were calculated from percentage inhibition of peak currents, evoked by a 200ms depolarization step from -60mV to +20mV and IC_{50} values were estimated by fitting the data to a logistic function by non-linear regression. Data are presented as means (95% Confidence limits of n observations).

The R(+) and the S(-) enantiomers as well as the combination of the two enantiomers (10nM-10μM), concentration dependently inhibited the peak calcium channel currents evoked by depolarization. The R(+) enantiomer was the least potent of the enantiomers with an IC_{50} in excess of 10μM (n=4). The S(-) enantiomer was considerably more potent with an IC_{50} of 290nM (200-800nM, n=4), whereas the combination of the enantiomers blocked the calcium channel currents with an IC_{50} of 330nM (200-400nM, n=4).

S(-) amlodipine is a potent calcium channel antagonist. Since the potency of the S(-) enantiomer alone is very similar to the potency of the combination of R(+) and S(-) enantiomers, we suggest that it is mainly the S(-) enantiomer that contributes to the therapeutic effect of amlodipine maleate.

This work was supported by Pfizer USA.
ADH is a British Heart Foundation Intermediate Fellow.

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412P CALCIUM STORE DEPLETION MAY UNDERLIE MUSCARINIC-INDUCED INHIBITION OF INTRACELLULAR CALCIUM RESPONSES TO BRADYKININ IN SH-SY5Y CELLS

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A range of cell surface receptors are linked, via GTP-binding proteins of the G_q family, to the increased activity of phospholipase C resulting in an enhanced rate of hydrolysis of phosphatidylinositol 4,5-bisphosphate. One consequence of this is the liberation of inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) and the subsequent release of calcium from intracellular stores. Given the number of cellular events influenced by calcium and its potential toxicity, limitation of intracellular calcium concentration ($[Ca^{2+}]_i$) in the face of prolonged exposure to a single agonist or simultaneous exposure to multiple agonists, may be a prerequisite for controlled cell function and survival. This study examined the interaction between muscarinic and bradykinin-mediated elevations in $[Ca^{2+}]_i$ in adherent populations of the human neuroblastoma cell line SH-SY5Y. Carbachol and bradykinin provoked rapid (approximately 10s) dose-dependent peak elevations in $[Ca^{2+}]_i$ measured using fura-2 with EC_{50}'s of (log_{10} M±s.e.mean) -6.255±0.159 (0.55μM) and -7.800±0.108 (16nM) respectively. In addition, carbachol produced a later (>60s) lower, sustained plateau phase with an EC_{50} of -6.643±0.218 (0.23μM). Maximal change in $[Ca^{2+}]_i$ at the peak in response to bradykinin was, however, only 69±12nM (n=18) (all mean±s.e.mean, n=3 unless stated) compared to 325±72nM in response to carbachol. At 130s following stimulation with a maximal concentration of carbachol (1mM) and in its continued presence, maximal bradykinin (1μM) was unable to provoke a change in $[Ca^{2+}]_i$. In contrast, bradykinin had no effect on the subsequent response to carbachol. At lower concentrations of carbachol (1μM and 0.1μM) there was a reappearance and increase of the bradykinin $[Ca^{2+}]_i$ response (50±8nM and 105±9nM respectively). Addition of atropine (10μM) but not EGTA (13mM) following maximal carbachol stimulation was also able to restore a full bradykinin response. Preincubation for 10min with a protein kinase C inhibitor (Ro-318220, 10μM) prior to carbachol stimulation failed to restore the response to bradykinin. In nominally calcium free conditions (approximately 2μM compared to 2mM), peak $[Ca^{2+}]_i$ responses to both carbachol and bradykinin showed reductions of 38% and 37% respectively while the sustained response to carbachol was severely attenuated (73% reduction). Under these conditions, stimulation with bradykinin elevated $[Ca^{2+}]_i$ by 54±6nM and markedly reduced the subsequent response to carbachol (36±3nM). In single cells, 18/20 cells responded to bradykinin and 20/20 to subsequent carbachol stimulation. Thus, carbachol and bradykinin access the same $Ins(1,4,5)P_3$-sensitive calcium-pool. Carbachol's ability to inhibit subsequent responses to bradykinin was independent of protein kinase C stimulation and most likely acts via the depletion of the calcium pool although a resensitization process dependent upon calcium entry can not be excluded. Bradykinin's inability to influence the response to carbachol in the presence of extracellular calcium probably relates to its comparatively small $[Ca^{2+}]_i$ elevation and the rapid and full desensitization of the response.

413P PHARMACOLOGICAL CHARACTERISATION OF THE HUMAN N-TYPE CALCIUM CHANNEL IN TRANSFECTED HEK 293 CELLS

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Williams et al. (1992) have expressed the N-type calcium channel ($\alpha 1_{B-1}$,α_{2b}, β_{1-2}) from human brain in HEK 293 cells, a continuous human kidney cell line which in the untransfected state has no measurable calcium current. We have have used transfected HEK 293 cells to characterize pharmacologically the human N-type calcium channel by both electrophysiological and fura-2 based microfluorimetric techniques.

Cells, plated on to poly-L-lysine coated coverslips and perfused with 5mM Ca^{2+}/TEACl-containing buffer were voltage-clamped and depolarized (Vh=-80mV to Vt=0 or +10mV) in the whole-cell configuration (Hamill et al., 1981). Depolarization evoked a rapidly activating, slowly inactivating calcium current, (range 150pA to 2nA) which coincided with a slowly developing and subsequently sustained rise in $[Ca^{2+}]_i$. The current exhibited marked sensitivity to Cd^{2+} with an IC_{50}=4.5±1.9μM (mean±SEM; N=3) which was rapidly reversible. Calcium current and flux into cells depolarized by high $[K^+]_o$ (70mM) was inhibited irreversibly by the cone snail toxins, ω-CgTX GVIA (Conus geographus) and ω-CmTX MVIIA (C. magus). Another toxin from C. magus, ω-CmTX MVIIC, displayed similar activity with regard to calcium current but differed from GVIA and MVIIA in that it was, at least, partially reversible. Conus toxins were distinguishable from each other by their speed of action, the rank order in terms of time to 100% block at 100nM being: MVIIC=fastest acting>MVIIA>GVIA=slowest acting. In contrast, ω-Aga IVA, a toxin from the American funnel-web spider Agelenopsis aperta, which has potent antagonist activity on the P-type calcium channel (Mintz et al., 1992) had no effect on calcium current or flux in these cells up to a concentration of 100nM. Flunarizine (1μM), a mixed-function calcium channel antagonist, caused 31±9% inhibition (mean±SEM; N=3) of the calcium current and 19±4% inhibition (mean±SEM; N=4) of the calcium flux into tranfected cells. However, neither nifedipine (1μM) nor verapamil (1μM) blocked the N-type calcium channels in these cells measured either fluorimetrically or electrophysiologically.

We conclude that this human calcium channel expressed in HEK 293 cells appears to have pharmacological properties similar to those of the native high voltage-activated N-type calcium channel.

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414P VOLTAGE- AND TIME-DEPENDENT INHIBITORY EFFECTS OF PROPAFENONE AND QUINIDINE IN VASCULAR SMOOTH MUSCLE

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Propafenone and quinidine are class I antiarrhythmic drugs which exert their therapeutic actions by a voltage- and frequency-dependent block of Na^+ channels. Recently, some class I antiarrhythmics have been reported to relax vascular smooth muscle by inhibition of Ca^{2+} entry (Carrón et al., 1991; Pérez-Vizcaíno et al., 1991). The present study was undertaken to study the voltage-dependence of the effects of quinidine and propafenone in rat aorta and porcine coronary artery.

Endothelium-denuded rat aortic and porcine left anterior descending coronary artery rings (3-4 mm in length) were mounted in Krebs solution for isometric tension recording under 2 and 0.5 g of tension, respectively (Pérez-Vizcaíno et al., 1991). The voltage-dependence of drug-induced effects was determined by incubating the rings in either normal (5 mM KCl, Ca^{2+}-free) or depolarizing Krebs solution (40 mM KCl, Ca^{2+}-free) and eliciting a contraction with a 100 mM KCl solution (2 mM Ca^{2+}) for 2 min (Godfraind & Salomone, 1991). The $^{45}Ca^{2+}$ influx was measured by the fraction of $^{45}Ca^{2+}$ uptake resistant to displacement by $LaCl_3$.

Both propafenone and quinidine, cumulatively added to the bath, relaxed, in a concentration dependent manner, the contractions induced by 80 mM KCl (IC_{50}s: 2.5 ± 0.1 x 10^{-6}M and 6.6 ± 0.7 x 10^{-6}M, n = 6, in aortic rings and 5.6 ± 0.6 x 10^{-6}M and 2.3 ± 0.2 x 10^{-5}M, n = 6, in coronary rings, respectively). In the rat aorta, propafenone (10^{-6}M and 3 x 10^{-6}M) and quinidine (3 x 10^{-6}M and 10^{-5}M), incubated for 90 min, inhibited the contractions induced by 80 mM KCl. The inhibition induced by quinidine increased as the time of depolarization was prolonged, reaching a steady state in 15-20 min. The inhibition induced by propafenone, in contrast, was not significantly time-dependent. The inhibitory effects of quinidine were accentuated when the drug was incubated in the depolarizing solution as compared to a normal solution (IC_{50}s 9.5 ± 1.5 x 10^{-6}M vs 1.6 ± 0.2 x 10^{-5}M, n = 7, $P < 0.05$ in aortic rings and 6.3 ± 1.6 x 10^{-6}M vs 1.6 ± 0.2 x 10^{-5}M, n = 7, $P < 0.05$ in coronary rings, respectively). In contrast, the effects of propafenone were not affected by the membrane potential (IC_{50}s 2.1 ± 0.1 x 10^{-6}M vs 2.7 ± 0.4 x 10^{-6}M, n = 8, $P > 0.05$ in aortic rings and 2.8 ± 0.3 x 10^{-6}M vs 3.5 ± 0.2 x 10^{-6}M, n = 7, $P > 0.05$ in coronary rings, respectively). Both 3 x 10^{-6}M propafenone and 10^{-5}M quinidine inhibited the 100 mM KCl-stimulated $^{45}Ca^{2+}$ uptake, the inhibition induced by quinidine (but not by propafenone) being significantly greater when incubated in depolarizing than in normal solution.

In conclusion, quinidine, as reported with some dihydropyridines, produces a voltage-dependent inhibition of contraction and Ca^{2+} entry in vascular smooth muscle suggesting a preferential binding of the drug to Ca^{2+} channels at depolarized potentials. Although the effects of propafenone were also mediated by Ca^{2+} channel blockade, they were not significantly affected by voltage.

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415P INHIBITION OF VOLTAGE-DEPENDENT Ca-CURRENT BY IMIDAZOLE DERIVATIVES IN INTESTINAL SMOOTH MUSCLE CELLS FROM THE GUINEA-PIG

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Imidazole antimycotics are potent inhibitors of cytochrome P450 enzymes (Ballard et al., 1988). This study describes the inhibitory action of some imidazoles on voltage-gated Ca channels in smooth muscle. Smooth muscle cells were freshly isolated from the longitudinal layer of guinea-pig ileum and Ca-current was recorded using whole-cell voltage-clamp. The external (bath) solution was based on NaCl and included 1.5 mM Ca. The internal (pipette) solution was based on CsCl and included 0.2 mM EGTA with no added Ca. Ca-current was elicited by stepping to 0 mV for 100 ms every 10 s from a holding potential of -60 mV.

Bath application of econazole caused inhibition of the Ca-current (K_d 490 nM, 21 cells) without obvious effect on the current-voltage relationship or on the rate of inactivation at 0 mV. Inhibition developed slowly (for 1 μM the exponential time constant was 371$\pm$84 s, mean$\pm$s.e.m., n=5; 21-24 °C) and was not readily reversed on wash-out of the drug. Clotrimazole had a similar effect (K_d 1.4 μM, 5 cells) but the polar imidazole derivatives dazmegrel (10 μM, n=2) and dazoxiben (10 μM, n=3; 100 μM, n=2) had no effect. Two inhibitors of cytochrome P450s that do not have an imidazole structure also inhibited the Ca-current: naphthoflavone (2 μM) gave 50% and 47% inhibition in 2 cells and metyrapone (100 μM) gave 19.4$\pm$16.5% inhibition (n=9). However, itraconazole (a triazole antimycotic) is an inhibitor of cytochrome P450s (Vanden Bossche et al., 1986) and yet it had no effect on the Ca-current at 10 μM (n=3), and bath application of a solution saturated with carbon monoxide also had no significant effect (n=11). The imidazoline derivative tolazoline inhibited the Ca-current but only at high concentrations (100 μM gave 30% and 23% inhibition in 2 cells).

These experiments have shown that two non-polar imidazole derivatives are potent inhibitors of voltage-dependent Ca channels in intestinal smooth muscle cells. This effect may be similar to that observed in GH_3 and chromaffin cells (Villalobos et al., 1992). The observations with itraconazole and carbon monoxide seem to suggest that the inhibition of Ca channels in smooth muscle cells might not result from inhibition of a cytochrome P450 but rather from some other interaction of these compounds either at a sight on the Ca channels or on a regulatory protein.

Dazmegrel and dazoxiben were kindly provided by Pfizer and itraconazole by Janssen. DJB is supported by the Wellcome Trust.

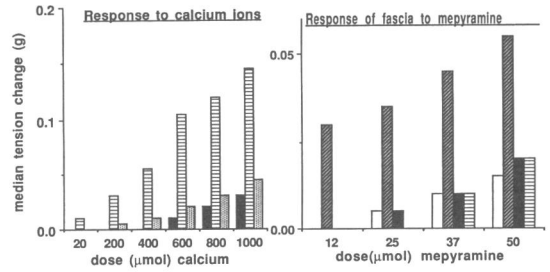
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416P THE *IN VITRO* REACTIVITY OF FASCIA FROM THE RAT AND GUINEA-PIG TO CALCIUM IONS AND MEPYRAMINE

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It has been suggested that fascia, superficial or deep, is simply connective tissue, which serves only supportive and mechanical force transmitting functions. Its most abundant cellular constituent, the fibroblast, has long been considered "non active" (Peer, 1955). This study investigated the in vitro reactivity of the fibroblast containing fascia obtained from the superficial and deep fascia to changes in the concentration of calcium ions and to mepyramine and compared their responses to those obtained from smooth and skeletal muscle.

Dorsal fascia(superficial and deep), vascular smooth muscle(aorta,n=6),and skeletal muscle(dorsal body wall,n=6) were obtained from female, Hooded Lister Rats(200-300g,Bradford strain) and male Dunkin Hartley guinea pigs(300-450g, Bradford strain). Tissue strips, 20x5mm, were superfused at 37°C using a flow rate of 2ml min-1 and a resting tension of 2g.Their contractility to $CaCl_2$ and the H1 antagonist, mepyramine were measured isometrically.



Both agents elicited concentration dependent, reproducible and reversible contractile responses (Fig 1) in both superficial (n=6) and deep fascia (n=6).In the guinea pig the deep fascia was significantly (P< 0.001,Mann Whitney U test) more sensitive to both agents then the superficial. Rat deep fascia was more sensitive(P< 0.05) to the calcium ions than the superficial. The aortae of the rat (P,< 0.05)and guinea pig(P< 0.001) were significantly less sensitive to calcium ions than the deep fascia of both species. Mepyramine relaxed the aortae at the concentration used. Skeletal muscle was unresponsive to both agents.

Key: Fascia -Superficial ; Rat ☐ GP ■ deep, rat ▨ GP ▤ aorta -rat ▥ GP ☐

These results show that cells present in rat and guinea pig fascia, both superficial and deep, are capable of contraction in vitro. The enhanced responsiveness of the deep fascia may reflect its greater fibroblast population. These contractions cannot be attributed to the presence of the vascular elements within the fascia. additionally they cannot be attributed to any skeletal muscle which was left adherent to the deep fascia.

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417P SOURCES OF Ca^{2+} IN NORADRENALINE AND ADENOSINE 5'-TRIPHOSPHATE (ATP)-EVOKED CONTRACTION OF RABBIT PROXIMAL SAPHENOUS ARTERY

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Nerve stimulation-evoked contractions of the rabbit proximal saphenous artery are mediated by co-released noradrenaline and ATP (Burnstock & Warland, 1987). Previous studies showed that the purinergic component was sensitive to the Ca^{2+} antagonist nifedipine, whereas the noradrenergic component was unaffected (Bulloch *et al.*, 1991). Furthermore, exogenous noradrenaline failed to stimulate phosphoinositide (PI) turnover, in contrast to the ATP analogue α,ß-methylene ATP (mATP; Nally *et al.*, 1992). The present study examined the role of intracellular and extracellular Ca^{2+} in noradrenaline, ATP and mATP-induced contraction of the rabbit proximal saphenous artery.

Segments of endothelium-denuded rabbit proximal saphenous artery were mounted in an organ bath containing warmed, oxygenated Krebs solution, and contractions recorded isometrically. Noradrenaline (0.03 - 100 μM; EC_{50} 1.10 μM, max. 1.64 ± 0.32 g wt) and ATP (30 μM - 10 mM; EC_{50} 630 μM, max. 1.21 ± 0.08 g wt) stimulated concentration-dependent contraction of the artery segments, while 10 μM mATP induced a mean contraction of 0.86 ± 0.13 g wt. The α_1-adrenoceptor antagonist prazosin (0.1 μM) caused a significant rightward shift of the concentration-response curve for noradrenaline (EC_{50} 13.50 μM). The α_2-adrenoceptor antagonist yohimbine (0.1 μM) had no effect (EC_{50} 1.05 μM). Nifedipine (1 μM) reduced the maximum noradrenaline-induced contraction by 43.3 ± 5.1 %, and contractions of artery segments bathed in Ca^{2+}-free Krebs solution were reduced by 89.6 ± 1.8 %. ATP-induced contractions were abolished by prior exposure of proximal saphenous artery segments to the P_2-receptor desensitizing agent mATP (10 μM) and the maximum contraction to ATP was reduced by 88.9 ± 4.1 % in Ca^{2+}-free Krebs solution. Nifedipine attenuated mATP-induced contractions by 50.0 ± 9.8 %. In experiments designed to assess the role of intracellular Ca^{2+} in contraction, artery segments were bathed sequentially in: A) normal Krebs solution (2.5 mM Ca^{2+}), B) Ca^{2+}-free Krebs solution for 5 min; and C) normal Krebs. Caffeine (10 mM)-induced contractions were not significantly different between the three conditions (A, 0.48 ± 0.13 g; B: 0.33 ± 0.13 g; C, 0.45 ± 0.08 g). In contrast, noradrenaline (3 μM)-induced contractions were attenuated in solution B, but were restored upon the re-addition of Ca^{2+} (A, 3.06 ± 0.63 g; B, 1.31 ± 0.44 g; C, 3.03 ± 0.61 g). Taken together, these results suggest that α_1-adrenoceptor-mediated contractions of rabbit proximal saphenous artery induced by exogenous noradrenaline involve both intracellular Ca^{2+} release and, in contrast to neurally-evoked noradrenergic contractions, influx of extracellular Ca^{2+} through voltage-sensitive Ca^{2+} channels. P_2-Purinoceptor-mediated contractions to ATP also involve influx of extracellular Ca^{2+}.

Supported by the Wellcome Trust.

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418P THE ACE INHIBITOR RAMIPRILAT AUGMENTS BRADYKININ-INDUCED CHANGES IN $[Ca^{2+}]_i$ IN BOTH BOVINE AORTIC AND ENDOCARDIAL ENDOTHELIAL CELLS

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Endocardial endothelial cells that line the chambers of the heart show distinct morphological differences to vascular endothelial cells (Brutseart and Andries, 1992). Whether these cells differ in their biochemical functions is not known. Stimulation of vascular endothelial cells by bradykinin (BK) is associated with an increase in intracellular calcium concentration ($[Ca^{2+}]i$). BK is degraded by angiotensin converting enzyme (ACE) and some of the therapeutically beneficial effects of ACE inhibitors may be related to their effects on BK metabolism. The purpose of this study was to compare the effects of BK (10^{-11} to 10^{-7}M) on $[Ca^{2+}]i$ in both bovine aortic endothelial (BAE) and bovine right ventricular endocardial (BRVE) cells and to investigate the effect of the ACE inhibitor ramiprilat ($3x10^{-6}$M) on these BK-induced changes.

BAE and BRVE cells were isolated as described previously (Smith and Lang, 1990 and Smith et al., 1991 respectively). Both cell types were used after sub-culturing onto fibronectin-coated glass coverslips for 4-5 days. $[Ca^{2+}]i$ was measured spectrophotofluorometrically after loading the cells with fura-2 AM essentially as described previously (Buchan and Martin, 1991). The basal $[Ca^{2+}]i$ for BAE (79.1±3.8nM, n=20) was significantly ($p<0.01$) lower than that for BRVE (95.4±3.6nM, n=25). Both cell types displayed significant ($p<0.01$) concentration-dependent, biphasic elevations in $[Ca^{2+}]i$, consisting of a large initial transient component followed by a lower relatively well sustained component. The initial peak Ca^{2+} response to the two highest concentrations of BK used (10^{-8} and 10^{-7}M) were significantly ($p<0.05$) greater in BRVE (490.5±25.9 and 510.7±29.9nM) than BAE (304.1±30.5 and 326.3±37.7nM) cells. Ramiprilat had no effect on the basal $[Ca^{2+}]i$ in either cell type. In BAE cells ramiprilat significantly ($p<0.05$) increased the BK-induced Ca^{2+} responses during both phases of the Ca^{2+} transient. In BRVE cells however, only the initial peak Ca^{2+} response to BK was augmented ($p<0.05$). These data show firstly that differences exist between BAE and BRVE cells both in their resting $[Ca^{2+}]i$ and the $[Ca^{2+}]i$ responses to exogenously added BK and secondly that ACE inhibition by ramiprilat augments BK responses in both cell types.

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419P DIHYDROPYRIDINE CALCIUM CHANNEL MODULATORS INHIBIT THE INDUCTION OF NO SYNTHASE AND PREVENT THE INHIBITION OF CELLULAR RESPIRATION CAUSED BY ENDOTOXIN IN J774.2 MACROPHAGES

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We have recently shown that nifedipine, but not verapamil or diltiazem, causes a concentration-dependent inhibition of the induction of nitric oxide (NO) synthase (NOS) by bacterial lipopolysaccharide (LPS) in the mouse macrophage cell line J774.2 and in cultured rat aortic smooth muscle cells (Szabó et al, 1993). Here we investigate in J774.2 cells (1) whether the inhibitory effect of nifedipine depends on the stimulus of induction and (2) whether other dihydropyridine (DHP)-type antagonists of calcium channels (nitrendipine, nimodipine, nisoldipine) or the calcium channel agonist BAY K 8644 also modulate the induction of NOS by LPS and (3) whether NO produced by J.774.2 cells inhibits their own cellular respiration, and whether DHPs can prevent this effect.

The mouse macrophage cell line J774.2 was cultured as previously described (Szabó et al, 1993). Nitrite production, an indicator of NO synthesis, was measured in the supernatant of J774.2 macrophages. Cell respiration, an indicator of cell viability, was assessed by the mitochondrial-dependent reduction of MTT to formazan. Conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline was measured in organ homogenates as described (Szabó et al, 1993). DHPs were gifts of Bayer AG Pharma Research Center (Wuppertal-Elberfeld, Germany). Stock solutions of the DHPs were made in DMSO; dilutions were made in culture medium. Student's unpaired *t*-test was used to compare means between groups. A *p*-value less than 0.05 was considered significant. Data are shown as means±sem.

In contrast to LPS-stimulated cells (1 μg/ml, 24h), where nifedipine dose-dependently inhibited nitrite production, when cells were stimulated with either LPS (1 μg/ml) plus γ-interferon (20 U/ml) or tumour-necrosis factor-α (300 U/ml) plus γ-interferon (20 U/ml), no inhibition of nitrite accumulation was observed. Pretreatment of J774.2 cells with other DHPs (10 nM - 3μM for 30 min) also inhibited the LPS-stimulated nitrite formation. For instance, at 1 μM, the inhibition was 59±3% for nitrendipine; 47±5% for nimodipine and 42±3% for nisoldipine (n=9; $p<0.05$). Interestingly, the DHP calcium channel agonist BAY K 8644 also caused a moderate, but significant inhibition of nitrite accumulation (by 16±3% at 0.1 μM, n=9; $p<0.05$). The inhibition of LPS-stimulated nitrite accumulation produced by nitrendipine, nimodipine or BAY K 8644 was significantly smaller when these agents were applied 2, 4 or 6h after LPS (n=9), indicating that they inhibit the induction, but not the activity of the induced NOS. The inhibitory effect of nisoldipine was not significantly reduced when the drug was applied 2, 4 or 6h after LPS (n=9). LPS (0.01 - 10 μg/ml) dose-dependently inhibited cellular respiration; this effect was prevented by N^G-methyl-L-arginine (3 mM). Moreover, the calcium channel blockers, at concentrations which inhibit NO production, also reduced the LPS-induced respiratory inhibition. For instance, nitrendipine (3 μM) reduced the LPS-induced (10 μg/ml) inhibition of cell respiration from 32±3% to 19±2% ($p<0.05$, n=9). DHPs (0.1 - 10 μM) did not directly inhibit the induced NOS activity in lung homogenates obtained from LPS-treated rats (n=4).

In conclusion, DHP antagonists and agonists of calcium channels inhibit the induction, but not activity of the calcium-independent isoform of NOS by LPS in J774.2 macrophages. This inhibitory effect is (1) dependent on the stimulus of induction, (2) related to the DHP structure, but not related to the modulation of intracellular calcium levels, and (3) is associated with prevention of the NO-mediated inhibition of mitochondrial respiration. (This work was supported by a grant of Glaxo Group Research Ltd. C.S. is supported by the Lloyds of London Tercentenary Foundation.)

Szabó Cs., Mitchell, J.A., Gross, S.S., Thiemermann C. & Vane, J. R. (1993) J. Pharmacol. Exp. Ther. 265, 674

420P CLONED HUMAN PARATHYROID HORMONE RECEPTORS MOBILISE CYTOSOLIC CALCIUM THROUGH A HEPARIN-INSENSITIVE MECHANISM

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(Introduced by D. Hoyer)

We have investigated calcium signalling induced by activation of cloned human parathormone (PTH) receptors. Following stable expression in HEK 293 cells (ca 120.000 receptors/cell), PTH was found to activate adenylate cyclase with an EC_{50} of 4.2 ± 0.46 x 10^{-10} M, but failed to stimulate phosphatidylinositol turnover. Despite undetectable inositoltriphosphate (IP_3) formation, PTH induced calcium release from intracellular stores. This study was undertaken to further characterize the PTH-induced calcium response.

HEK 293 cells were cultured on glass coverslips in DMEM/F12 medium supplemented with 10 % foetal calf serum. The cells were voltage clamped at -50 mV and Fura-2 AM (5 μM; 30 min) was loaded into the cells before electrode impalement. Changes in cytosolic calcium were measured in single cells using a photodiode array connected to a fluorescence microscope as described previously (Boddeke et al., 1993). The data are presented as mean values ± s.e.mean.

PTH induced a concentration-dependent ($3x10^{-8}$ - 10^{-7} M) increase in cytosolic calcium which was not affected after removal of extracellular calcium. The EC_{50} of PTH was 7.2 ± 0.5 x 10^{-8} M and the maximal increase of cytosolic calcium, attained at 10^{-7} M was 580 ± 82 nM (n = 12). A similar mobilization of intracellular calcium (maximal increase = 630 ± 71 nM; n = 14) in HEK 293 cells was observed after carbachol-induced (10^{-4} M) activation of endogenously expressed M_1 muscarinic receptors, which couple to phospholipase C. Inclusion of the IP_3 receptor antagonist heparine (10^{-4} M) in the pipette solution did not affect PTH-induced (10^{-7} M) mobilization of cytosolic calcium and a maximal increase of 541 ± 43 nM (n = 8) was observed. In contrast, the calcium response to carbachol (10^{-4} M) was reduced by 86 ± 7 % (n = 11) after intracellular application of heparin (10^{-4} M).

We conclude that PTH mobilizes intracellular calcium via a heparine insensitive pathway.

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421P EVIDENCE FOR A SINGLE INTRACELLULAR CALCIUM STORE MOBILISED BY NORADRENALINE AND CAFFEINE IN RAT ISOLATED MESENTERIC ARTERIES

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It is well established that the release of Ca^{2+} from an intracellular store mediates at least in part the contractile response of smooth muscle to agonists. In large arteries there is evidence that noradrenaline (NA) and caffeine (CAF) release Ca^{2+} from different intracellular Ca^{2+} stores (Kanaide *et al*, 1987), however this is not necessarily true for resistance arteries. Ryanodine (RY) is a plant alkaloid that depletes the intracellular Ca^{2+} store. Thapsigargin (TSG), a putative selective Ca^{2+}-ATPase inhibitor has been shown to prevent repletion of the intracellular Ca^{2+} store (Low *et al*, 1991).

Isolated rat mesenteric arteries (<500μm diameter, n=16) were mounted in a myograph (Mulvany & Halpern, 1977) to study the releasable calcium fraction sensitive to NA and CAF. Arteries were contracted by exposure to KPSS (118mM K^+), NA(10μM), CAF(10mM). Responses in physiological saline (PSS: 2.5mM Ca^{2+}) and Ca-free PSS (+1mM BAPTA) before and after RY (10μM,1h) and TSG (3μM,30min) were compared. We also studied the effect of RY and TSG on the Ca^{2+} concentration-response relationship (100nM-10mM) under depolarising conditions (Ca-free KPSS). In some experiments intracellular Ca^{2+} (Ca_i) was measured using fura-2. Arteries were loaded with 6μM fura-2AM for 3h (n=6). Following washout of fura-2AM fluorescent emission at 510nm was measured using 340nm and 380nm excitation. The ratio of these 340:380nm signals was used as a measure of Ca_i.

Table — Response to stimulants before and after treatment.

	NA PSS(%)		NA Ca-free PSS(%)		Caffeine Ca-free PSS(%)		KPSS(N/m)		n
	before	after	before	after	before	after	before	after	
RY	92±5	91±7	39±6	1±0*	14±3	0±0*	4.6±0.2	4.5±0.2	6
TSG	102±2	94±13	23±5	0±0*	7±1	1±0*	5.1±0.3	5.1±0.4	4

*Responses are % response to KPSS before treatment ± s.e. mean. * indicates p<0.05 by Wilcoxon Signed Rank Test.*

Both RY and TSG virtually abolished contractile responses to NA and CAF in Ca-free PSS. The transient Ca_i rise induced by NA and CAF was also abolished by RY and TSG. In contrast, force and Ca_i responses to KPSS and NA in PSS appeared little affected. The Ca^{2+} concentration-response curve showed no shift after RY (log EC_{50} -2.60±0.59 before, -3.48±0.28 after, n=6) and TSG (log EC_{50} -3.50±0.06 before, -3.25±0.11 after, n=4). In rat mesenteric arteries the intracellular Ca^{2+} store contributes little to the overall contractile response, when extracellular Ca^{2+} is present.

This work was supported by a British Heart Foundation grant.

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422P THE EFFECT OF CALCIUM CHANNEL ANTAGONISTS ON THE MAMMALIAN NEUROMUSCULAR JUNCTION

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Voltage dependent calcium channels (VDCCs) have been shown to play an essential role in controlling neurotransmitter release within the central nervous system (Mulkey and Zucker, 1991).
A range of selective neuronal VDCC antagonists have been studied on the neuromuscular junction of mice using the isolated phrenic nerve diaphragm preparation (Braga et al., 1991).
Cadmium, a non-selective VDCC antagonist reduced in a concentration-dependent manner, the twitch tension elicited by electrical stimulation of the phrenic nerve (IC_{50}= 56±6μM, n=5).
Omega agatoxin IVA (Aga IVA) a potent and selective P-type VDCC antagonist also caused a concentration and time dependent block of the response to phrenic nerve stimulation (IC_{50}=60±4nM, n=5), but did not affect the increase in tension due to direct muscle stimulation. The block caused by Aga IVA was readily reversed by washing and could also be partially reduced by the addition of physostigmine (5μM).
Interestingly synthetically prepared authenic FTx (a toxin isolated from the funnel web spider) a reported P-type antagonist (Llinas et al., 1992) failed to affect the preparation even at relatively high concentrations(100μM).
Omega conotoxin (CgTx) MVIIC a preferential Q-type VDCC antagonist (Zhang et al.,1993) failed to affect the twitch response to phrenic nerve at levels (0.1μM)reported to be selective for Q-type VDCCs but did affect the preparation at higher levels (38±17 % reduction at 1μM, n=3).
CgTx GVIA an N-type VDCC antagonist failed to affect the responses to phrenic nerve stimulation even at the relatively high concentration of 1μM.
The results indicate that an Aga IVA sensitive, and possibly P-type channel, is the major functional VDCC in the neuromuscular junction in the mouse and presumably acts by regulating neurotransmitter release.

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Key words:-calcium channel antagonists neuromuscular junctions

423P MATIOTOXIN STIMULATES CALCIUM INFLUX IN HL-60 CELLS THROUGH A PATHWAY DISTINCT FROM RECEPTOR-ACTIVATED NON-SELECTIVE CATION CHANNELS

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Maitotoxin, a marine toxin from *Gambierdiscus toxicus*, increases the cytosolic free calcium concentration ($[Ca^{2+}]_i$) in many cell types by stimulation of calcium entry (Gusovsky & Daly, 1992). The mechanism whereby maitotoxin stimulates calcium entry is still uncertain. Soergel *et al.* (1992) have recently suggested that maitotoxin activates non-selective cation channels (NSC channels). The aim of the present study was to characterise the maitotoxin-induced calcium entry in HL-60 cells, a cell line devoid of voltage-dependent calcium channels and with a well characterised NSC channel (Krautwurst *et al.*, 1992).

Table 1 Effects of SKF 96365, Gd^{3+} and Cd^{2+} on stimulus-induced calcium entry

		Stimulus-induced calcium entry (% control)	
		Maitotoxin	fMLP
Blocker			
SKF96365	1μM	67 ± 2*	91 ± 4
SKF96365	10μM	26 ± 10*	47 ± 2
Gd^{3+}	10μM	113 ± 9*	9 ± 8
Cd^{2+}	10μM	59 ± 21*	98 ± 11

Data shown are means ± SD of three independent observations. Mean calcium entry was 214 ± 112 nM for fMLP and 998 ± 499 nM for maitotoxin. * Significantly different from fMLP ($p < 0.05$, t-test)

Stimulation of dibutyryl cyclic AMP differentiated HL-60 cells with the chemotatic peptide *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP; 0.1 μM), the Ca^{2+}-ATPase inhibitor thapsigargin (0.1 μM) or maitotoxin (25 ng/ml) resulted in an increase in $[Ca^{2+}]_i$ as measured by fura-2 fluorescence (Schwaner *et al.*, 1992). Unlike fMLP and thapsigargin, maitotoxin produced no increase in $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}. Both fMLP and maitotoxin-induced increases in $[Ca^{2+}]_i$ were inhibited by the NSC channel blocker 1-{β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenylethyl}-1*H*-imidazole hydrochloride (SKF 96365) in a concentration-dependent manner. However, the maitotoxin-induced increase in $[Ca^{2+}]_i$ was more sensitive to inhibition by SKF 96365 than the fMLP-induced increase (Table 1). fMLP-induced increases in $[Ca^{2+}]_i$ were blocked by cations with Gd^{3+} (10 μM) being more effective than Cd^{2+} (10 μM), whereas for maitotoxin Cd^{2+} was more effective than Gd^{3+} (Table 1). In the presence of extracellular Mn^{2+} both fMLP and thapsigargin stimulated quenching of fluorescence in fura-2 loaded HL-60 cells. In contrast maitotoxin produced no Mn^{2+}-induced fluorescence quenching, suggesting that the maitotoxin mechanism is Mn^{2+} impermeant.

Taken together these results suggest that maitotoxin does not stimulate the non-selective cation channel activated by fMLP, but instead activates calcium influx by a different mechanism.

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This work was supported by funds from the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie. IFM is a Australian National Health and Medical Research Councils C.J. Martin Fellow.

424P ENFORCEMENT OF TETANIC CONTRACTION PREVAILS IN BIPHASIC EFFECT INDUCED BY ADINAZOLAM

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Muscle weakness, becoming a special point of concern due to the introduction of potent benzodiazepines, often given in high doses before surgery, has prompted further search for derivatives with less serious neuromuscular blocking effects. In previous studies, findings of others (Driessen et al., 1984) with regard to biphasic actions of benzodiazepines on twitch and tetanic contraction were confirmed (Eigenhuis et al., 1990). It was shown, moreover, that both pre- and postsynaptic mechanisms are involved in the enhancement followed by inhibition of isolated twitch and tetanic contractions. Benzodiazepines furthermore cause a voltage- and frequency dependent block of endplate potentials of skeletal muscles (Van Wilgenburg et al.,1993). In this study effects of the new benzodiazepine adinazolam on respiratory skeletal muscle have been compared to the biphasic effect of diazepam on tetanic contraction.

Isolated phrenic nerve-diaphragm preparations from female rats (150-200 gram), kept in Krebs-Ringer solution (37°C) and gassed with 5% CO_2 in oxygen, were stimulated supramaximally (0.1 Hz and 10 to 50 Hz, duration 0.2 msec) either indirect or direct. In the latter case preparations were paralysed with pancuronium and stimulated directly between a surgical steel wire attached to the tendon and to pins onto which the costal margin was impaled. Bath concentrations of diazepam and adinazolam varied between 0.01 and 0.6 mM (n=6).

Diazepam and adinazolam both induce a concentration related enhancing effect followed by a blocking action on twitch and tetanic contraction upon indirect stimulation. At a concentration of 0.01 mM adinazolam enhances the tetanic contraction at 40 and 50 Hz to 158±6%(SEM) and diazepam to 150±4% of the controls. These effects are sustained during subsequent contractions. Maximal increasing effects of 462±69% on tetanic contractions are obtained for diazepam (0.1 mM, 30 Hz) and 615±92% for adinazolam (0.3 mM, 30 Hz). These effects, however, can not be sustained. A complete blocking effect, finally, occurs at 0.3 mM diazepam and 0.6 mM adinazolam respectively. The effects on indirect evoked twitches are also biphasic but less pronounced as compared to the tetanic contractions. At three times higher concentrations comparable effects are found upon direct stimulation. In contrast to the former experiments these effects are not reversible.
In conclusion, both diazepam and adinazolam produce enhancement of tetanic contractions at relatively low concentrations, and use- and frequency-dependent neuromuscular blocking effects at higher concentrations. The blocking potency of adinazolam is less as compared to that of diazepam. It is suggested therefore that adinazolam might be more safe when muscle weakness must be avoided.

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425P RELAXATION RESPONSES OF HUMAN UTERINE AND SPONTANEOUSLY HYPERTENSIVE RAT FEMORAL ARTERIES TO PARATHYROID HORMONE, HISTAMINE AND ACETYLCHOLINE

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Serum parathyroid hormone (PTH) levels were increased in preeclamptic women (Belizan et al., 1988).Changes by acetylcholine (ACh) in the renal vascular resistance in humans with essential hypertension were increased as compared to those in normotensive subjects (Hollenberg and Adams ,1976). In the present study ,relaxation responses of human uterine arteries and spontaneously hypertensive rat (SHR) blood vessels to PTH (1-34) were compared to those to histamine (HA) and ACh. Helical strips of aortae and femoral arteries from 10-week-old male Wistar Kyoto rats (WKY) and SHR and uterine arteries from patients undergoing abdominal hysterectomy were suspended under optimal resting tensions (aortic and uterine 1 g, femoral 500mg) in physiological salt solution (Fujimoto and Matsuda, 1991). The blood vessels were carefully removed to protect the integrity of the endothelial lining. To observe relaxation, the strips were contracted with $3x10^{-6}$M (uterine) or 10^{-6}M noradrenaline (aortic and femoral) in the presence of $2x10^{-6}$M propranolol. HA was more potent as a vasorelaxant in the uterine artery than in the aorta and femoral artery (Table 1). Potencies (-log EC_{50}) of HA in the SHR blood vessels were similar to those in the WKY tissues. The potencies of ACh in the aorta and femoral artery were larger than in the uterine artery. There was significant difference between WKY and SHR in EC_{50} values for ACh-induced relaxation of the femoral artery. PTH(10^{-10}-10^{-7}M) did not relax the uterine artery. PTH relaxed more markedly the SHR than the WKY aortae, and the potency of PTH was similar in the femoral arteries from WKY and SHR. It was suggested that in preeclamptic women, an increase in serum PTH levels did not contribute to a decrease in uterine arterial tensions. The greater sensitivity of the rat aorta to PTH might be caused by hypertension.

Table 1. EC_{50} values for histamine-, acetylcholine-, parathyroid hormone-induced relaxations

	Uterine artery	Thoracic aorta		Femoral artery	
		WKY	SHR	WKY	SHR
Histamine($x10^{-6}$ M)	0.17±0.01	9.1±1.6	6..1±1.1	15.2±3.0	10.9±2.0
Acetylcholine($x10^{-8}$ M)	25.8±7.75	2.2±0.3	2.2±0.2	0.53±0.04	0.80±0.10*
Parathyroid hormone ($x10^{-8}$ M)	NR	3.7±0.7	1.4±0.3*	5.0±1.6	3.1±0.4

Data are expressed as means ±s.e.m.(n=6-14). NR; no relaxation observed. * $p<0.05$ compared to WKY. An analysis of variance with Student-Newman-Keuls test was used for the statistical analysis.

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426P EXOGENOUS L-ARGININE REVERSES IMPAIRMENT OF EDRF-DEPENDENT COLLATERAL PERFUSION IN A RABBIT MODEL OF HYPERCHOLESTEROLAEMIA

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We have recently shown that EDRF-dependent collateral flow after arterial occlusion is severely impaired in rabbit model of hypercholesterolaemia (Randall *et al.*, 1993). We have now investigated the effects of addition of the substrate for EDRF synthesis, L-arginine, on collateral flow in hypercholesterolaemia.

Rabbits were fed on either a normal diet (n=13) or a diet containing 1% cholesterol (n=13) for 8 weeks. The rabbits were killed, the central ear artery cannulated and the ear perfused *in vitro* with oxygenated physiological salt solution containing 10μM indomethacin and 5% (w/v) dextran. Following arterial occlusion the level of collateral perfusion was assessed by X-ray microangiography as filling of a segment of the central ear artery, ligated at its midpoint and apex (Randall *et al.*, 1993). In some preparations 10mM L-arginine was added to the perfusion fluid 15 mins prior to arterial occlusion. Statistical comparisons were made by Student's t-tests.

In controls segmental filling 2 mins after occlusion was 19.5±4.9% (mean±s.e.mean) of the preocclusion value and rose to 34.5±6.7% after 60 mins. In the hypercholesterolaemic rabbits the level of filling 2 mins after occlusion was 10.5±3.6% and peaked at 19.5±4.6% at 5 mins, after which segmental filling fell to ca. 6-10% and remained at this level and was significantly less ($P<0.01$) than in the controls. In the hypercholesterolaemic preparations that were pretreated with L-arginine segmental filling 2 mins after occlusion was 15.8±5.0% but increased over the remainder of the experiment to 30.9±9.6% at 90 mins when it was significantly ($P<0.01$) greater than in the preparations from cholesterol fed rabbits not treated with L-arginine. Pretreatment of control preparations (n=9) with L-arginine did not influence collateral perfusion which was 35.6±8.4 at 60 mins.

The results indicate that the impairment of EDRF-dependent collateral perfusion in hypercholesterolaemia can be reversed by exogenous addition of L-arginine.

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427P INDAPAMIDE BINDING IN GUINEA PIG VASCULAR SMOOTH MUSCLE CELLS

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Indapamide is a "thiazide-like" diuretic which lowers blood pressure by a combined diuretic and direct vascular action. The indapamide binding site in guinea pig renal membranes has the properties of a carbonic anhydrase (Calder *et al*, 1992). This study has investigated [^{3}H]indapamide binding in guinea pig vascular smooth muscle cells (VSMC) and its displacement by other drugs.

Guinea pig mesenteric arteries were carefully dissected free, cleaned and cut into small pieces (explants) and placed into tissue culture flasks. This tissue was cultured using minor modifications of the explant technique of Chamley Campbell *et al* (1979) in Dulbecco's medium containing 25mM HEPES buffer supplemented with 10% fetal calf serum (FCS), penicillin (100μg/ml) and streptomycin (100μg/ml). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. VSMC migrated out from the explants to form a monolayer which was subcultured by trypsinisation. VSMC were seeded, 250μl/well, in 96 well plates and allowed to attatch overnight. The standard 10% FCS medium was replaced with serum free medium to growth arrest the cells. Binding studies were performed in quadruplicate. Each well contained 100μl solution containing serum free medium, [^{3}H] indapamide (120nM) and drugs in buffer. The plates were left on ice for 4h and the cells were then rapidly washed three times with cold saline solution. The wells were filled with NaOH solution (1M) to dissolve the cells and left overnight, counts were recorded on a scintillation counter.

The effects of different diuretics on [^{3}H]indapamide binding is illustrated in table 1.

Table 1. Effects of drugs on [^{3}H]indapamide binding

Drug :	Indapamide	Acetazolamide	Hydrochlorothiazide	Metolazone	Cicletanine	Nicardipine
IC_{50} (μM) :	1.25(±0.8%)	0.25(±0.8%)	340(±0.9%)	230(±0.2%)	N/D	N/D

N/D means no displacement (up to 100μM). N=4; values are in μM (± % error)

This study suggests that the [^{3}H]indapamide binding site in guinea pig VSMC has the properties of a carbonic anhydrase. Acetazolamide has been reported to produce cerebral vasodilatation through inhibition of membrane bound carbonic anhydrase (Allen, 1983) it is possible that such inhibition may participate in the vasorelaxant properties of indapamide.

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428P EFFECTS OF ATRIOPEPTIN ON AQUEOUS HUMOUR FORMATION AND ON CYCLIC GMP IN CULTURED CILIARY EPITHELIUM OF THE BOVINE EYE

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Atriopeptin lowers intraocular pressure (IOP) in vivo (rabbit) and in the isolated bovine eye. Evidence that this is due to a decrease in aqueous humour formation (AHF) is scanty and reported increases in ciliary levels of cyclic GMP do not localize this effect to the secretory epithelium. We now confirm that atriopeptin directly reduces AHF and that its receptors are present on ciliary epithelial cells.

Bovine eyes were arterially perfused with modified Krebs' solution and AHF rate (given as $K_{out}.10^{-4}.min^{-1}$) was monitored fluorimetrically by perfusion (flow rate 2.25ml. min^{-1}) of the anterior chamber with an aqueous humour substitute containing fluorescein (Wilson et al., 1993). Atriopeptin or vehicle was injected into the arterial perfusate in a bolus of 5μl. For tissue culture, the ciliary processes were partially digested by perfusing the isolated eye first with DMEM (25-30min) then with sterile Ca^{2+}-free buffer (pH 7.4) containing EDTA (0.25mM) for 12min and, finally, with collagenase (0.1%) in a sterile buffered saline for 20min. After dissection, sheets of ciliary epithelium were harvested, washed and cultured in DMEM containing both foetal and newborn calf sera (10% of each) and gentamycin (200μg.ml^{-1}). Cells were passaged at confluence (7 to 8days). Cyclic nucleotides were measured using RIA kits (DuPont) and protein by the Lowry method.

Atriopeptin (50 pmol) decreased K_{out} from 47±3 (n=8) to 29±2 (n=8) over a 60min perfusion. When incubated with a beta-adrenoceptor agonist, ciliary tissue shows a dose-dependent rise in cyclic AMP: the increase due to terbutaline (10^{-6}M) in ciliary processes cut from fresh eyes was 50±16%, while that in cultured cells was 75±9% (basal cyclic AMP levels in pmol: 9.4±0.9, n=6 and 49±3, n=12, resp.) confirming that cultured cells still express beta-adrenoceptors. Incubation (6min) of the cultured cells with atriopeptin produced dose-dependent increases in cyclic GMP as shown in Table 1.

Table 1. Effect of atriopeptin on cyclic GMP levels (fmol.mg^{-1} protein; n=9) in cultured ciliary epithelium

Treatment	First passage cells	Second passage cells
Control	64 ± 7	67 ± 7
Atriopeptin, 10^{-6} M	174 ± 13 ***	141 ± 18 **
10^{-7} M	135 ± 18 **	78 ± 8
10^{-8} M	66 ± 5	-

Significance of difference from control (t-test): ** P<0.01; *** P<0.001.

These results indicate that atriopeptin acts on guanylate cyclase-linked receptors located on the ciliary epithelium and hence reduces IOP by a direct effect on ciliary function. The culture of ciliary epithelium from bovine eyes offers a model for study of the subcellular mechanisms of drugs used in the treatment of glaucoma..

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429P EFFECT OF THROMBIN INHIBITORS, ANTI COAGULANTS AND A FIBRINOLYTIC AGENT ON THROMBIN-INDUCED CEREBRAL EMBOLISM IN RABBITS

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Sustained accumulation of ^{111}Indium-labelled platelets within the cranial vasculature of rabbits following intracarotid (i.c.) injection of thrombin (Thr) can be prevented by infusion of thrombin inactivators and anticoagulants (Liu et al., 1993). We have now studied the effects of bolus injection of thrombin inactivators, anticoagulants and a fibrinolytic agent, given before or after Thr, on the platelet accumulation response . ^{111}In-labelled platelets were monitored in the cranium of anaesthetised NZW rabbits as described previously (May et al., 1990). Responses to i.c. injection of Thr (90u kg^{-1}) were calculated as the area under the curve (AUC) of % change from baseline levels against time (0-2 h) and presented as mean ± s.e.mean. The significance of differences was assessed by analysis of variance followed by a modified t-test.

D-Phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK; 10-30μg kg^{-1}) 1 min before Thr produced dose-related reductions in the response from 7443±743 (n=7) to 56±34 (n=4; $p<0.01$) with 30μg kg^{-1}. 1 min after Thr, PPACK (100μg kg^{-1}) had no significant effect (control: 8558±878, treated: 9575±596, both n=4). Hirulog (1mg kg^{-1}) 1 min after Thr had no inhibitory effect (n=4). Unfractionated heparin (UFH; 25-100u kg^{-1}) 1 min before Thr reduced the response from 8851±804 (n=5) to 1373±260 (n=4; $p<0.01$) at 100u kg^{-1}. This dose of UFH 1 min after Thr produced a significant ($p<0.05$) increase from 5837±1031 (n=4) to 9838±1227 (n=4). The low molecular weight heparin, Fragmin (150u kg^{-1}) 1 min before Thr reduced the response significantly ($p<0.05$) from 6793±1548 to 1777±686 (both n=4) whereas when given 1 min after Thr, there was no significant effect (control: 6077±542, treated: 7532±1122; both n=4). Recombinant tissue plasminogen activator (rt-PA; 1-15μg kg^{-1}) 1 min before Thr reduced the response from 8301±1492 (n=6) to 357±274 (n=3; $p<0.01$) with 15μg kg^{-1}. 1 min after Thr, rt-PA (15μg kg^{-1}; n=4) still significantly ($p<0.01$) reduced the response from 6883±759 to 2480±637.

These data show that the inhibitory effects of thrombin inactivators and anticoagulants are much reduced or absent when treatment is delayed until 1 min after injection of Thr whereas the effect of the fibrinolytic rt-PA,though reduced, is still highly significant.

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430P VITAMIN K INHIBITS CHEMOTAXIS AND ACTIN POLYMERIZATION IN HUMAN POLYMORPHONUCLEAR NEUTROPHIL LEUKOCYTES

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Vitamin K_1 has been reported previously to have an anti-inflammatory effect in rat paw oedema due to carrageenan (Eichbaum *et al*, 1979). Vitamin K_3, an analogue lacking the C_3 alkenyl side-chain, has been reported to inhibit several functions of polymorphonuclear neutrophil leucocytes (PMNs), such as superoxide production and shape change in response to a chemotaxin (Gallin *et al*, 1982). We examined the effects of vitamins K_1 and K_3 on the chemotaxis of PMNs which is a principal component of inflammation. Exposure of PMNs to a chemotaxin such as n-formyl-methionyl-leucyl-phenylalanine (FMLP) induces shape change and alignment of the cells along the chemotactic gradient. Chemotaxis involves contraction of actin-containing microfilaments, and sequential re-alignment of the cytoskeleton. We have therefore also considered the effects of vitamins K_1 and K_3 on actin polymerization.

PMNs were isolated from human whole blood by dextran sedimentation of the erythrocytes and discontinuous percoll gradients. Cells were resuspended in HEPES-buffered Hanks balanced salts solution, pH 7.4, + 0.1%(w/v) bovine albumin. PMNs ($2x10^6ml^{-1}$) were loaded into the upper wells of Boyden chambers, and 100nM FMLP placed in the lower wells. The cells were allowed to migrate through 140μm cellulose nitrate filters along the chemotactic gradient for 1h. After fixing and staining, the leading front cells were counted (Wilkinson, 1988). Neutrophils ($5x10^6ml^{-1}$) were exposed to either FMLP, complement fragment C5a, or platelet activating factor (PAF), and the quantity of polymerized actin determined by staining with NBD-phallacidin and analysis on the fluorescence activated cell sorter (FACS) (Joseph & MacDermot, 1992).

Treatment of PMNs for 2h with a maximum concentration (≥10μM) of vitamin K_1 inhibited chemotaxis by 41 ±0.5% (n=8), with half this effect at 2.0 ±0.6μM. Treatment with ≥20μM vitamin K_3 completely halted cell movement (n=12), with IC_{50} = 2.0 ±0.1μM. Actin polymerization in response to 100nM FMLP was stimulated 2.5 ±0.5 fold (n=15) compared to control. Maximum concentrations (≥10μM) of vitamin K_1 inhibited actin polymerization by 62 ±12% (n=6), with IC_{50} = 1.7 ±sem <0.1μM. Vitamin K_3 (≥50μM) inhibited actin polymerization by 97.6 ±0.3% (n=6), with IC_{50} = 5.5 ±0.5μM. Comparable effects of vitamins K_1 and K_3 were also observed when the cells were exposed to 5nM C5a or 10μM PAF.

We conclude that vitamin K_1 or K_3 has a significant effect on chemotaxis in neutrophils by prevention of actin polymerization.

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431P A PRELIMINARY STUDY ON THE EFFECTS OF BRADYKININ AND RELATED COMPOUNDS ON ISOLATED MOUSE UTERUS

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The purpose of this study was to investigate the response of the isolated mouse uterus to bradykinin (BK), Lys-BK and des-Arg^9-BK and to examine the potential role of prostaglandins in this response using indomethacin.

To synchronise oestrus mature female BKW mice were pretreated with stilboestrol (s.c. 100μg 100g^{-1}) 18h prior to the study. Uterine horns were superfused (2ml min^{-1}) with oxygenated Krebs solution (Sangha & Senior, 1989). After equilibration of the tissue agonists were injected directly into the flow of the superfusate, as bolus doses. As the profile of spontaneous activity and sensitivity to agonists often changed throughout the course of the experiments, comparisons were made between preparations in a non-paired manner. Because of the variations in myogenic activity results have been normalised to take into account the level of background activity and are expressed as ratios of test response (T) to background activity (B), these results can then be expressed as $ED_{1.5}$ (excitatory dose) values, i.e. the dose required to evoke a response one and a half times that of a background, spontaneous contraction (see Senior *et al.*, 1991). The presence of indomethacin (2.79 μM) in the perfusate had no effect on myogenic activity.

The responses to BK (0.0005-10nmol) and Lys-BK (0.001-10nmol) and des-Arg^9-BK (0.005-10nmol) were all qualitatively similar, in that they each evoked monophasic contractions (n = 7 in all experiments). The dose response curves to BK and Lys-BK were bell shaped and, as can be seen from the table below, the responses (max T/B) elicited by des-Arg^9-BK and Lys-BK were weak when compared to BK. The inclusion of indomethacin in the perfusate abolished the bell-shaped dose-response curves to BK and Lys-BK.

Kinin	In the absence of indomethacin		In the presence of indomethacin	
	Mean $ED_{1.5}$ (nmol)	Max T/B (s.e. mean)	Mean $ED_{1.5}$ (nmol)	Max T/B (s.e. mean)
BK	0.0013	11.6 ± 4.5	0.0005	17.7 ± 7.3
Lys-BK	0.1	2.8 ± 0.7	0.01	4.8 ± 2.0
des-Arg^9-BK	0.28	2.3 ± 0.9	0.28	1.8 ± 0.3

The results of this preliminary study suggest that the mouse uterus does possess kinin receptors, predominantly of the B_2 type. It would also seem that activation of the B_2 receptor initiates the release of cyclooxygenase products which have an inhibitory effect on myogenic activity.

Sangha, R.K. & Senior, J. (1989) *Br. J. Pharmacol.* 98, 762P.
Senior, J., Marshall, K., Sangha, R., Baxter, G.S. & Clayton, J.K. (1991) *Br. J. Pharmacol.* 102, 747-753.

432P CHARACTERISATION OF THE THROMBOXANE (TP) RECEPTOR POPULATION IN ISOLATED MOUSE UTERUS

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A previous study in this laboratory (Knights *et al.*, 1992) has shown that the response of the isolated mouse uterus to PGE_2 and PGE analogues is similar to that found in the human (Senior *et al.*, 1991), in that the mouse, unlike many other species, also contains an EP-receptor mediating inhibition of myogenic activity. The fact that mouse, like human uterus, has a heterogeneous EP-receptor population led us to suggest that it may be a suitable *in vivo* model for further investigation of prostaglandins on the female reproductive tract. The aim of this functional study was, therefore, to characterise another prostaglandin receptor, the TP-receptor, on mouse uterus to see if this also corresponds to the human. In this work the stable TX mimetic U46619 was used in conjunction with the following TP-receptor antagonists: BAYu3405; BM 13.505; GR32191; SQ29548 (Norman *et al.*, 1992).

To synchronise oestrus mature female BKW mice were pretreated with stilboestrol (s.c., 100μg 100g^{-1}) 18h prior to the study. Uterine horns were superfused (2ml min^{-1}) with oxygenated Krebs solution containing 2.79μM indomethacin (Sangha & Senior, 1989). After equilibration of the tissue agonists were injected directly into the flow of the superfusate, as bolus doses (n = 6). As the profile of spontaneous activity and sensitivity to agonists often changed throughout the course of the experiments, comparisons were made between preparations in a non-paired manner. Because of the variations in myogenic activity results have been normalised to take into account the level of background activity, thus the results have been expressed as T/B ratios of 'test' (T) agonist responses as a ratio of background (B) contractions (see Senior *et al.*, 1991).

The stable TP-receptor agonist, U46619, evoked a purely contractile response, stimulation of the tissue was marked, consisting of a rapid increase in baseline tonus on which was superimposed frequent phasic contractions. All the thromboxane antagonists tested caused a progressive rightward displacement of the dose response curve to U46619 with increasing concentration of antagonist.

Thromboxane antagonist	Dose range	pA_2	(range)
BAYu3405	10^{-9}-10^{-6}M	9.1	(8.9-9.3)
BM13.505	10^{-9}-10^{-6}M	8.5	(8.1-8.8)
GR32191	10^{-9}-10^{-6}M	7.5	(7.0-7.9)
SQ29548	10^{-9}-10^{-6}M	8.6	(8.0-9.0)

The results obtained from this study indicated that the mouse uterus does possess receptors for thromboxane. The pA_2 values calculated from this study are similar to those obtained by Clayton *et al.* (1991) on non-pregnant human uterus. Therefore, in terms of both EP- and TP-receptors the mouse may represent a suitable *in vivo* model for studies involving the uterus. This study is currently being extended to cover the other PG receptors present on the mouse uterus.

Clayton, J.K., Marshall, K., Sangha, R.K. & Senior, J. (1990) *Br. J. Pharmacol.* 102, 172P.
Knights, J., Marshall, K. & Senior, J. (1992) *Br. J. Pharmacol.* 107, 416P.
Norman, P., Cuthbert, N.J., McKenniff, M.G. & Gardiner, P.J. (1992) *Eur. J. Pharmacol.* 229, 171-178.
Sangha, R.K. & Senior, J. (1989) *Br. J. Pharmacol.* 98, 762P.
Senior, J., Marshall, K., Sangha, R., Baxter, G.S. & Clayton, J.K. (1991) *Br. J. Pharmacol.* 102, 747-753.

433P THE EFFECT OF Des-Arg^9 (Leu^8) BK (A B_1 RECEPTOR ANTAGONIST) ON THE ACTIONS OF BRADYKININ (BK) AND RELATED COMPOUNDS ON ISOLATED HUMAN MYOMETRIUM

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Previous preliminary studies in this laboratory have shown that BK and related compounds are potent agonists on human myometrium. BK and Lys-BK result in a biphasic response whereas des-Arg^9-BK produces a monophasic response of contractile nature on isolated human myometrium (Abbas *et al.*, 1993). The aim of this study was to characterise the response of human myometrium, using BK, Lys-BK and des-Arg^9-BK, in terms of B_1 receptor involvement.

Samples of human myometrium were obtained from pre-menopausal patients at hysterectomy (NP) or from pregnant patients (P) during Caesarean section (all patients gave written consent). The myometrial strips were set up for superfusion using Krebs' solution containing indomethacin 2.79μM (37°C, 95% O_2/5% CO_2) at 2ml min^{-1} as described by Senior *et al.* (1991). After equilibration of the tissue agonists were injected directly into the flow of the superfusate, as bolus doses. As the profile of spontaneous activity changed throughout the course of the experiments, comparisons were made between preparations in a non paired manner. Because of the variations in myogenic activity results have been normalised to take this into account and are expressed as ED_1 (excitatory) values (nmol), i.e. the dose required to evoke a response equivalent to a background contraction (see Senior *et al.*, 1991). Des-Arg^9 (Leu^8)BK (3 x 10^{-7}M) was added to the superfusate at least 1h before administration of agonists, it exerted no effect on the spontaneous activity of the tissues at this dose level.

The responses to BK (0.001-50nmol) and Lys-BK (0.001-50nmol) were biphasic and consisted of an initial increase in myometrial tension followed by inhibition of myogenic activity. The presence of des-Arg^9 (Leu^8)BK did not exert a significant effect on the responses to BK and Lys-BK (n = 5 in all experiments).

Kinin	ED_1 values (nmol)			
	NP		P	
	alone	+ des-Arg^9 (Leu^8)BK	alone	+ des-Arg^9(Leu^8)BK
BK	0.002	0.07	0.008	0.02
Lys-BK	0.08	0.32	0.08	0.25
des-Arg^9-BK	0.02	2.50	0.02	1.00

The presence of des-Arg^9 (Leu^8)BK in the superfusate significantly attenuated (P<0.01-0.001) the monophasic contractile response to des-Arg^9-BK (0.01-50nmol). The standard error associated with each response was within 10% of the mean.

The results suggest that the response to des-Arg^9-BK may be mediated by specific receptors, probably of B_1 type, whereas the responses to BK and Lys-BK are not mediated through B_1 receptors at a significant level.

Senior, J., Marshall, K., Sangha, R., Baxter, G.S. & Clayton, J.K. (1991) *Br. J. Pharmacol.*, 102, 747-753.
Abbas, F., Marshall, K. & Senior, J. (1993) *Br. J. Pharmacol.* 110, 154P.

434P BACTERIAL LIPOPOLYSACCHARIDE INDUCES *DE NOVO* SYNTHESIS OF CYCLO-OXYGENASE ACTIVITY IN FRESHLY ISOLATED HUMAN MONONUCLEAR CELLS

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At least two distinct forms of the enzyme cyclo-oxygenase (Cox) are known to exist. Cox 1 is a constitutive enzyme whereas Cox 2 is induced by a variety of factors, such as bacterial lipopolysaccharide (LPS) and interleukin 1 (Xie et al., 1992). Almost all previous reports on Cox induction have utilised adherent cells. In the present study we have examined the ability of LPS and arachidonic acid (AA) to affect Cox activity in freshly isolated human mononuclear cells.

Human blood, collected from volunteer donors, was layered onto methylcellulose (1%)/hypaque (34%) to remove red blood cells. The mononuclear cells were isolated (Böyum, 1968) and resuspended in culture medium, RPMI1640 to give a final concentration of 10^6 cells ml^{-1} (93.9±0.5% lymphocytes and 4.6±0.5% monocytes; n=16). The ability of AA or LPS (E. Coli) to stimulate thromboxane (Tx)B_2 production was measured at 37°C, using 0.4x10^6 cells in a total volume of 0.5ml. Reactions were terminated by centrifugation and samples of the supernatant stored at -20°C. Prostaglandins were measured using enzyme immunoassay. Cells were incubated with the inhibitors for 4 hours and 50 mins prior to the addition of AA, or for 1 hour prior to the addition of LPS. Exposure to AA or LPS was for 10 mins or 4 hours respectively. All results are mean ± s.e.m. (n=3-6) and are expressed as percentage increase over basal. The average basal release of TxB_2 was 273 ± 62 and 633±152pg per million cells for 10min and 24hr incubation times respectively.

Exogenous AA (0.01-1μM) produced a rapid, concentration related increase in TxB_2 output (3498±1081% at 1μM after 10 min). LPS (0.001-10μgml^{-1}) produced a slow, time dependent accumulation of TxB_2, with a lag phase of 2 hours. Peak cumulative production of TxB_2 was observed 24 hours after addition of LPS (10μgml^{-1}; 604±205%). LPS also produced a small increase in PGE_2 levels but had no effect on 6-keto $PGF_{1\alpha}$ levels. Pre-incubation of cells for 1 hour with aspirin (300μM) followed by washing, prevented the increase in TxB_2 production seen on addition of exogenous AA (1μM). However, following removal of aspirin, LPS (10μgml^{-1}) was still able to produce a large increase in TxB_2 (572±197%). Dexamethasone (1μM), cycloheximide (10μM) and actinomycin D (10μM) produced no significant inhibition of TxB_2 release following addition of exogenous AA (1μM, 10 min) i.e. 10.1±4.5, 3.0±1.1 and 8.1±4.1% respectively. In contrast, LPS stimulated (10μgml^{-1}) TxB_2 production was markedly inhibited by pretreatment with dexamethasone (70.7±9.8%), cycloheximide (98.2±1.3%) and actinomycin D (94.8±2.9%).

The differences in time course and the effects of transcription and translation inhibitors, actinomycin D and cycloheximide, suggest that TxB_2 production by AA is attributable to the provision of substrate for an existing enzyme, whilst LPS induces de novo synthesis of cyclo-oxygenase.

Böyum, A. (1968). Scand. J. Lab. Invest., **21**, 77-89.
Xie, W., Robertson, D.L. and Simmons, D.L. (1992). Drug Dev. Res., **25**, 249-265.

435P *MYO*-INOSITOL-1,4,6-TRISPHOSPHOROTHIOATE: AN INHIBITOR OF INOSITOL TRISPHOSPHATE-INDUCED $^{45}Ca^{2+}$ RELEASE

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Myo-inositol 1,4,5-trisphosphate ($InsP_3$) is a widely recognised second messenger responsible for the mobilisation of Ca^{2+} from non-mitochondrial intracellular stores upon receptor activation. Recent developments in inositol chemistry have facilitated the investigation of the $InsP_3$ receptor by the use of a variety of synthetic analogues as probes (Potter 1990). We describe here the properties of a number of inositol phosphate analogues in permeabilised rabbit platelets. Rabbit platelets were isolated and washed according to Murphy *et al* (1991). The platelets (10^9/ml) were suspended in an "intracellular like" buffer containing 5 mM ATP then permeabilised with saponin (40 μg/ml) for 1 min. The washed platelets were then loaded with ^{45}Ca for 60 min. and stimulated with inositol phosphates or analogues for 3 min. at room temperature, then the remaining cell associated ^{45}Ca determined by rapid filtration. Of the twenty compounds we examined most were equally efficacious in their ability to release ^{45}Ca (75-85% maximal release) but with varying potencies, $InsP_3$ having an EC_{50} of 1.14 $\pm$0.19μM (mean $\pm$ s.e., n=6). However of significant interest were three phosphorothioate analogues; *scyllo*-inositol-1,2,4,5-tetrakisphosphorothioate (*scyllo*-$InsPS_4$), L-*chiro*-inositol-2,3,5-trisphosphorothioate (L-*chiro*-$InsPS_3$) and *myo*-inositol-1,4,6-trisphosphorothioate [Ins(1,4,6)PS_3] which showed varying degrees of partial agonism giving maximal ^{45}Ca release of 61.7$\pm$ 11% (n=3), 42.3$\pm$ 6% (n=3) and 21.2$\pm$.4% (n=3) respectively (see figure 1). In marked contrast, the corresponding phosphates to each of these compounds showed full agonism, eg the *scyllo*-$InsP_4$ and L-*chiro*-$InsP_3$ showed EC_{50} of 0.1 $\pm$0.05μM (n=3) and 1.9 $\pm$0.1μM (n=3) respectively. Of the three phosphorothioate analogues only Ins(1,4,6)PS_3 exhibited antagonism at the $InsP_3$ receptor, co-incubation of Ins(1,4,6)PS_3 (300nM -100μM) with 300nM $InsP_3$ at 0°C produced a dose dependent inhibition of $InsP_3$-induced ^{45}Ca release, such that 100μM Ins(1,4,6)PS_3 produced approximately 50% inhibition, figure 2.

Fig.1.

Dose response curves to $InsP_3$, *scyllo*-$InsPS_4$ & *chiro*-$InsPS_3$

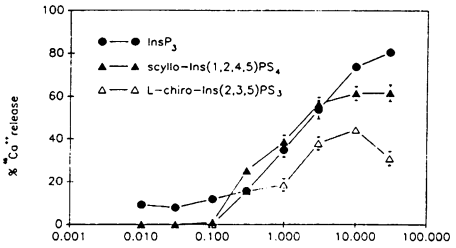
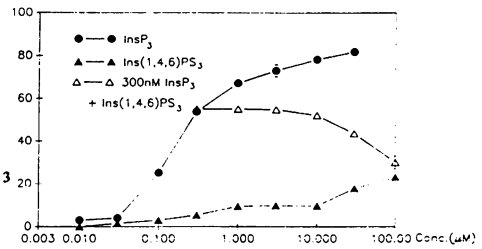


Fig.2

Dose response curves to $InsP_3$, Ins(1,4,6)-PS_3 & co-incubation of $InsP_3$ & Ins(1,4,6)PS_3



We have described the first inositol phosphate analogue which acts as an antagonist at the $InsP_3$ receptor in platelets, namely Ins(1,4,6)PS_3. Although L-*chiro*-$InsPS_3$ is a weak antagonist at the $InsP_3$ receptor in SH-SY5Y neuroblastoma cells (Safrany *et al* 1993), this activity was not present in platelets. Thus, these results support the concept of tissue specific receptor sub-types and provide a new lead compound for antagonist design.

Murphy,C.T., Elmore,M. ,Kellie,S. and Westwick,J. (1991) Biochem.J., 278, 255-261.
Potter,B.V.L. (1990) Nat.Prod.Rep., 7, 1-24.
Safrany,S.T., Willcox,R.A., Liu,C., Dubreuil,D., Potter,B.V.L. and Nahorski,S.R. (1993) Mol.Pharmacol., 43, 499-503.
This work is supported by SERC and the Wellcome Trust. BVLP is a Lister institute fellow.

436P COLLAGEN-INDUCED ST SEGMENT ELEVATION IN ANAESTHETIZED RATS: EFFECTS OF ASPIRIN, DIPYRIDAMOLE AND CALCIUM ANTAGONIST DRUGS

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Large clinical studies (e.g. Physician's Health Study, 1989) have demonstrated the primary role played by platelets in the genesis of ischaemic heart disease. The aim of the present study was to characterise a simple, new screening test in "vivo" in which the effects of various drugs against ischaemia, induced by platelet aggregation were assessed. Two consecutive i.v. doses of 1 mg/kg collagen (C1 and C2) were given 15 min apart to groups (n=6) of pentobarbitone-anaesthetized rats and drugs or vehicle given i.v. 5 min before C2. The ST segment changes from leads I, II and III electrocardiograms were measured, scored and meaned (mST), plotted against time and drug-induced anti-ischaemic effects quantified by comparing the areas (A) under the mST/time curves in control and drug-treated animals. Arterial blood pressure (BP) and heart rate (HR) were also measured. In control animals C1 maximally increased mST from 0 to 1.33 ± 0.13 units, decreased BP from 165 ± 4 to 112 ± 6 mmHg and decreased HR from 449 ± 5 to 375 ± 10 beats/min, effects which recovered within 10 min. Responses to C2 were more marked (mST, BP and HR were 1.81 ± 0.13 units, 73 ± 10 mmHg and 298 ± 22 beats/min respectively). The antiaggregant drugs, dipyridamole (D) (0.3 and 1 mg/kg) and aspirin (Asp) (10 mg/kg) significantly reduced A ($p < 0.05$) from control values of 15.4 ± 1.6, 15.4 ± 1.6 and 22.7 ± 2.1 to 9.4 ± 1.4, 6.1 ± 1.4 and 9.9 ± 2.3 units respectively in the absence of direct effects on BP or HR. Asp also antagonised the haemodynamic effects of collagen whilst D did not . The calcium antagonists verapamil (0.1-1 mg/kg) and diltiazem (0.3-1 mg/kg), both reduced BP (from 172 ± 4 to 93 ± 4 and from 164 ± 7 to 98 ± 6 mmHg respectively at 1 mg/kg) and HR (from 484 ± 6 to 273 ± 23 and from 432 ± 8 to 366 ± 22 beats/min respectively) but did not modify either the ischaemic or haemodynamic effects of collagen. The $5HT_2$ antagonist, ketanserin (0.3-1 mg/kg) either alone or in combination with diltiazem (0.3 ± 0.3 mg/kg) similarly failed to modify any of the effects of collagen.
These results suggest that this test may be used to detect compounds which prevent platelet-linked ischaemia. However, $5HT_2$ receptors do not appear to play a role. In addition, vasodilatation and bradycardia mediated by calcium antagonism do not prevent ischaemia in this model. In conclusion, evaluation of collagen-induced ST changes in rats appears to be a useful and simple technique to detect anti-ischaemic activity mediated via platelet antiaggregatory actions.

The Steering Committee of the Physician's Health Study Group, (1989). N. Engl. J. Med. 312, 129-135.

437P AN INTERACTIVE COMPUTER SIMULATION OF THE SQUID GIANT AXON PREPARATION FOR TEACHING UNDERGRADUATE STUDENTS

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Traditionally, opportunities for active learning in physiology and pharmacology have taken the form of student practical classes often involving live animal preparations. In response to rising costs many universities have integrated interactive, computer-based simulations into their curricula, many of which may be used to replace traditional undergraduate laboratory experiments. Here we present details of a computer simulation program which simulates the squid giant axon preparation. It is suitable for advanced undergraduate students and allows them to learn, by investigation, the ionic basis of resting and action potentials.

The program is written in C++ (Borland) to run on any IBM-compatible microcomputer with a graphics adaptor (VGA, EGA, Herc). A combination of text and high-resolution, colour graphics is used to describe the preparation, the apparatus and the experimental method used for voltage and current clamping of the axon. Interactive sections of the program allow the student to simulate:

Investigation of the resting potential: the student may select, from a menu, to vary the concentrations of sodium, potassium and chloride ions in the extracellular fluid and axoplasm and record changes in the resting potential of the membrane. Results, which are derived from the Goldman-Hodgkin-Katz model, are displayed on a simulated oscilloscope screen on the monitor from which students are able to take measurements.

Investigation of the action potential - Current and Voltage Clamping: students design experimental protocols from a series of menus which allow them to: vary the ion concentrations of sodium, potassium and chloride ions; set protocols for electrically stimulating the axon; investigate threshold and refractory period; investigate sodium and potassium currents using voltage clamp techniques (including the effects of *tetrodotoxin* and *tetraethylammonium*). Results, derived from widely accepted algorithms (Hodgkin & Huxley, 1952), are presented on a simulated oscilloscope screen on the monitor.

A companion program *Nerve Biophysics Tutorials* aimed at first year undergraduate students has been previously demonstrated to the Society (Brown *et al.*, 1993). This work was supported by The Lord Dowding Fund (NAVS).

Hodgkin, A.L. & Huxley A.F. (1952) *J. Physiol.*, **117**, 500-544.
Brown, E.R., Dewhurst, D.G. & Williams, A.D. (1993) *Brit. J. Pharmac. Proc. Suppl.* (in press).

438P A COMPUTER SIMULATION OF EXPERIMENTS TO DEMONSTRATE THE EFFECTS OF PHARMACOLOGICAL AGENTS ON THE CUTANEOUS INFLAMMATORY RESPONSE IN THE ANAESTHETISED RABBIT

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We present details of a computer simulation of a range of experiments designed to demonstrate the action of inflammatory mediators and pharmacological agents on the *in vivo* inflammatory response in the anaesthetised rabbit. The program uses data obtained from actual experiments and is aimed at undergraduate students of pharmacology. It may be particularly useful for teaching students either to support laboratory practicals or, in those departments where lack of equipment and/or technical expertise precludes this, as a student-centred alternative.

The program, which is contained on a single diskette, is written in C++ (Borland) for IBM-compatible microcomputers running a range of graphics drivers (EGA, VGA). **Introduction and Methods** sections combine text and high-resolution colour graphics to describe the animal preparation used in the experiments, the methods employed to measure oedema formation (extravascular accumulation of ^{125}I - albumin) and inflammatory cell (neutrophil) accumulation, and to provide the student with the essential background information required to understand the inflammatory response. The main section of the program is **Experiments** which allows the student to select, from a menu, to study the effects of the following agents on oedema formation (and where appropriate on neutrophil numbers) in normal rabbits:

(1) a range of direct mediators of increased microvascular permeability [histamine, bradykinin, platelet activating factor (PAF), Substance P, leukotriene D_4], either alone (dose-response relationships), in the presence of a vasodilator (prostaglandin E_2) or in combination with receptor antagonists;

(2) a range of agents which cause inflammation principally via neutrophil accumulation [complement Factor C5a, cytokines interleukin-1 and interleukin-8, the bacterial peptide f-methyl-leucyl-phenlalanine (FMLP), leukotriene B_4, Tumour Necrosis Factor (TNF_{alpha})], either alone (dose-response relationships) and in the presence of a vasodilator (prostaglandin E_2). The effects of neutrophil depletion and the importance of adhesion molecules are also covered;

(3) non-steroidal (local and systemic effects) and steroidal anti-inflammatory agents.

Results are presented in graphical form either as bar-charts or line graphs.

The program is accompanied by printed support materials and may be used in association with a teacher-designed protocol or for independent learning.

439P DEMONSTRATION OF A WINDOWS-BASED PROGRAMME FOR MULTIPLE CHOICE QUESTIONS

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Graphic user interfaces such as Windows 3.1 provide a user friendly environment in which to run applications. Furthermore they provide a standard across several PC configurations. The programme was written and compiled using Visual Basic 3.0

A bank of questions can be chosen using the Load File menu. Questions are presented in turn (Next button) and the student responds by clicking the appropriate True, Don't know or False (T,?,F) buttons (Figure 1). Clicking the Finished button prevents further input and allows the student to see the correct answers by running through the questions again. The marks (1 for correct, -1 for incorrect) for each question and the running total are displayed. Clicking the box containing the correct answer a window containing short explanation of the topic is displayed. Context sensitive help is available.

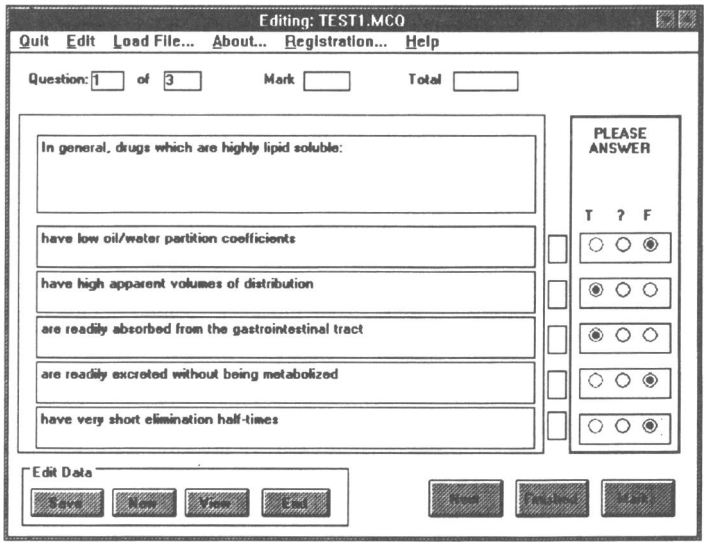


Figure 1. Example of the screen in edit mode. Students use the same screen but the edit buttons are not visible.

The questions are entered using the Edit menu. Once the appropriate password has been entered, an additional set of command buttons: Save, New, View and End, are provided for editing. In the edit mode, the questions are typed into the appropriate boxes and the correct answers recorded using the T F buttons. Each question is saved as a record in a random access data file, so additional questions can be added at any time. The usual Cut, Copy and Paste editing commands are available, allowing text to be transferred within and between questions and from other applications concurrently running under Windows. Evaluation copies are available from the authors.

440P INFLEXION: AUTOMATED ANALYSIS OF RADIOLIGAND BINDING DATA WITH MICROSOFT EXCEL

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Radioligand binding forms an essential part in the development of many pharmaceutical compounds, either during screening of novel chemicals, or as part of the profiling their pharmacology. Historically, radioligand binding experiments were small scale, with each scientist studying perhaps one or two types/classes of receptors, performing a single investigation per day. Today, the situation in many pharmaceutical companies is quite different. Radioligand binding is now extremely automated, involving the use of robotic liquid handling systems for drug dilution and assay set-up, and filtration apparatus capable of harvesting up to 96 samples at a time. One result of this increase in efficiency, is a vast increase in results produced by each scientist and their subsequent analysis is becoming the rate-limiting step in assay throughput. We have developed a system of analysis within Microsoft Excel, called INFLEXION, which is capable of coping with this volume of information.

The software requirements are Microsoft Windows (version 3.1) and Microsoft Excel (Version 4.0). The system can be run on IBM compatible PC's (at least 386 specification) or on the Macintosh making it suitable for most computer users. Although Excel was originally developed as a business package, version 4.0 was improved to include scientific capabilities. In order to analyse radioligand binding data, it was necessary to develop a system of iterative curve fitting within this package, as it does not contain such a defined function. This was achieved though the use of the Solver function within Excel which allows iterative analysis of linked formulae. The Solver function was programmed to minimise the mean square difference between actual experimental values and those predicted by a four parameter logistic equation. This allowed the analysis of inhibition curves, producing an estimation of the inhibition constant of a drug as well as the maximum and minimum binding and Hill coefficient for each curve.

Efficient analysis of data is achieved through the combination of direct transfer of data from gamma or scintillation counters, and a series of linked spreadsheets in Excel which sort and analyse the data according to the assay design. A set of macros (programmable code within Excel) control data processing and provide the flexibility required by the use of different assay formats. INFLEXION can process data from inhibition curves composed of any number of Total, Non-Specific and Specific observations. In addition, up to 240 protocols and dilution sets describing the assay design for individual experiments can be generated, stored and edited. Currently, INFLEXION can analyse and produce reports on 32 inhibition curves in about 15 minutes with minimal user input. Consequently, raw data (DPM) can be efficiently converted to inhibition constants within a single software package. Although we have used this system for analysis of radioligand binding data, in essence any equation can be fitted to experimental results making it suitable for the analysis of the variety of data types produced by scientists.

It is hoped that the demonstration of this approach to data analysis will help pharmacologists develop their own custom applications for the routine processing of results. INFLEXION is demonstrated using data from a radioligand binding experiment performed using cloned receptors.

441P 5-HT_{1D} AUTORECEPTORS MODULATE STIMULATED 5-HT RELEASE IN THE GUINEA-PIG DORSAL RAPHÉ NUCLEUS

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442P PLATELET-DERIVED NITRIC OXIDE IN HYPERTENSIVE AND NORMOTENSIVE CONTROL SUBJECTS

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Nitric oxide (NO) is generated by the endothelium and produces relaxation of adjacent vascular smooth muscle. In addition, NO inhibits platelet adhesion and aggregation. In 1990, Radomski *et al.* demonstrated that washed human platelets can produce NO,and attenuate platelet aggregation, when stimulated by a variety of aggregating agents. At variance with this work, Mollace *et al.* (1991) were unable to show that platelet derived NO modulates platelet aggregation. In addition, intra-arterial administration of N^G-monomethyl-L-arginine (L-NMMA) to healthy subjects does not potentiate *ex vivo* or *in vitro* collagen induced platelet aggregation (Vallance *et al.* 1992). Hypertensive patients appear to exhibit impaired endothelium-dependent relaxation and reduced vasoconstriction in response to L-NMMA, suggesting an impairment of NO production. We examined the role of platelet NO synthesis in regulation of aggregation to the physiological agonist adenosine diphosphate (ADP) in blood from untreated hypertensive patients (HT) and age and sex matched normotensive controls (NT) using L-NMMA to inhibit NO production. We hypothesised that inhibition of NO production would enhance ADP induced aggregation in NT and that this effect would be reduced in platelets from HT.

Platelet aggregation was measured by the method of Born (1962) in a Malin 6 channel aggregometer interfaced with a Macintosh computer. In these studies, subjects were 10 untreated HT and 10 age and sex matched NT controls. Blood was collected via a 19 G needle into acid citrate dextrose (9 ml blood into 1 ml: citric acid, 8 mg/ml; sodium citrate 22 mg/ml; glucose, 20 mg/ml) and centrifuged for 10 min at room temperature at 120 *g* to obtain platelet rich plasma (PRP). The remaining blood was spun for a further 20 min at 3000 *g* to obtain platelet poor plasma. (PPP) ADP was used as the aggregating agent (2.5-100 μM) either in the presence of vehicle (0.9 % saline) or L-NMMA (25 μM). The course of aggregation was studied for 10 min, and both maximum extent (the percentage of the difference between PRP and PPP) and initial rate of aggregation were calculated. Statistical analysis was by repeated measures analysis of variance.

ADP produced dose dependent aggregation in both NT and HT, and the aggregation profile was no different between groups. Although L-NMMA tended to increase rate and extent of aggregation in both groups, this effect was not statistically significant.

Conc	Extent of aggregation , %(±Sem)				Rate of aggregation,% min^{-1},(±Sem)			
ADP	HT		NT		HT		NT	
μM	Vehicle	L-NMMA	Vehicle	L-NMMA	Vehicle	L-NMMA	Vehicle	L-NMMA
2.5	34.3 (±7.6)	40.3 (±8.6)	35.2 (±5.7)	43.3 (±8.0)	31.3 (±3.2)	32.0 (±2.6)	30.1 (±3.6)	32.9 (±6.5)
5	49.8 (±7.4)	54.2 (±7.0)	52.5 (±6.5)	51.0 (±6.7)	36.6 (±2.2)	38.9 (±3.1)	33.0 (±3.2)	33.4 (±3.2)
20	60.8 (±5.0)	64.0 (±4.7)	62.8 (±4.7)	67.3 (±4.4)	40.5 (±1.8)	40.7 (±2.3)	34.5 (±3.4)	36.8 (±3.0)
100	68.9 (±4.6)	71.7 (±4.7)	68.0 (±4.4)	72.1 (±2.8)	40.3 (±4.6)	42.9 (±2.1)	37.7 (±3.3)	36.9 (±2.8)

Our results are consistent with those of Mollace *et al.* (1991) and Vallance *et al.* (1992). However, although there was no statistically significant effect of L-NMMA, there did appear to be a trend for potentiation of aggregation by L-NMMA. It is unlikely that this small effect is of functional significance and impaired production of NO in platelets from HT was not demonstrated. Platelet NO may play a more important physiological role in inhibition of platelet/platelet adhesion rather than aggregation.

[This work was supported by a grant from the Scottish Home and Health Department]

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443P LIPOTEICHOIC ACID, A CELL WALL COMPONENT OF GRAM POSITIVE BACTERIA, CAUSES HYPOTENSION AND INDUCTION OF NITRIC OXIDE SYNTHASE IN ANAESTHETISED RATS

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An enhanced formation of nitric oxide (NO) by the inducible nitric oxide synthase (iNOS) contributes importantly to the circulatory failure (hypotension and vascular hyporeactivity to vasoconstrictor agents) in endotoxin (lipopolysaccharide, LPS) shock (Thiemermann, 1994) and to the vascular decompensation in haemorrhagic shock (Thiemermann *et al.*, 1993). However, there is little information regarding the pathophysiological importance of NO in gram-positive sepsis. Here, we investigate the effects of lipoteichoic acid (LTA) from gram-positive bacteria, previously shown to induce nitric oxide synthase in vascular smooth muscle *in vitro* (Auguet *et al.*, 1992), on (i) blood pressure, (ii) vasoconstrictor responses to noradrenaline (NA), and (iii) induction of iNOS in anaesthetised rats.

Male wistar rats (199 - 268 g) were anaesthetised with sodium thiopentone (120 mg kg^{-1}, i.p.). The carotid artery was cannulated for the measurement of haemodynamic parameters, and the jugular vein for the administration of compounds. LTA (10 mg kg^{-1}, i.v., n = 5) or saline (n = 4) was administered intravenously at time 0 and blood pressure and heart rate were recorded for 360 min. The pressor response to NA (1 μg kg^{-1}, i.v.) was determined 15 min prior to and every 60 min after the injection of LTA. The NA responsiveness was calculated as the area under the curve and expressed as mmHg min. At the end of the experiment the rats were killed by i.v. injection of air and the lung, spleen, liver and brain were frozen in liquid nitrogen. The activity of iNOS was determined in homogenates of the organs by the conversion of ^{3}H-L-arginine to ^{3}H-L-citrulline in the absence of calcium as described previously (Thiemermann *et al.*, 1993).

Injection of LTA resulted in a decrease of the mean arterial blood pressure (MAP) from 121 ± 2 mmHg to 99 ± 3 mmHg within 60 min ($P < 0.01$). A further fall in MAP was observed commencing at approximately 150 min after injection of LTA to 75 ± 2 mmHg at 240 min. After 360 min, the MAP in LTA-treated rats was 73 ± 2 mmHg compared to 102 ± 10 mmHg in saline-treated rats ($P < 0.01$). Simultaneous injection of LTA and polymyxin B (0.05 mg kg^{-1}, i.v.), which binds LPS, did not prevent the fall in MAP elicited by LTA alone (79 ± 3 mmHg, 240 min, n = 3). In addition, LTA attenuated the pressor response elicited by NA at 60 min from 32 ± 5 mmHg min in saline-treated rats to 16 ± 2 mmHg min in LTA-treated rats ($P < 0.05$). Similarly, at 360 min the NA responsiveness was attenuated significantly by LTA from 46 ± 8 mmHg min to 20 ± 3 mmHg min ($P < 0.05$). At 360 min after injection of LTA, there was a significant increase in iNOS activity (in pmol L-citrulline 30 min^{-1} mg^{-1} protein) in lung (191 ± 55 vs. 12 ± 6), spleen (74 ± 10 vs. 8 ± 7) and liver (50 ± 14 vs. -1 ± 14). In contrast, no increase in iNOS activity was observed in brain homogenates (18 ± 14 vs. 9 ± 6).

Thus, in anaesthetised rats, LTA causes (i) a sustained hypotension, (ii) a reduction of the pressor responses elicited by NA, and (iii) an induction of iNOS activity. These results suggest that an enhanced formation of NO by iNOS contributes to the circulatory failure associated with gram-positive sepsis.

This work was supported by a grant from Glaxo Research Group Ltd. SJDK is a recipient of a fellowship from the European Communities.

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444P COMPARISON OF THE CARDIOVASCULAR RESPONSES TO CHRONIC AND ACUTE TREATMENT WITH L-NITROARGININE METHYL ESTER IN THE RAT

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L-nitroarginine methyl ester (L-NAME) is a potent inhibitor of nitric oxide synthase (NOS). Chronic oral treatment with L-NAME causes a sustained rise in blood pressure accompanied by profound changes in renal architecture (Gardiner et al., 1990; Ribeiro et al., 1992). Here we have compared the effect of long term oral treatment with L-NAME to the effects of L-NAME administered acutely on the blood pressure of the rat and the *ex-vivo* responses of the isolated perfused kidney (IPK) and isolated thoracic aorta (RTA).

Male Wistar rats (220-250g) were dosed with L-NAME (approx. 70mg/kg/day) by addition to the drinking water for 4-6 weeks. After induction of anaesthesia (thiopentone (Intraval); 120 mg/kg i.p.), the carotid artery and jugular vein were cannulated. After 30 min for stabilization the cardiovascular responses to bolus i.v. doses of sodium nitroprusside (SNP, 10nmol/kg) or acetylcholine (ACh, 1nmol/kg) were recorded (n=5-7). The renal artery was cannulated and the kidney isolated and perfused at a constant rate (10ml/min) with Krebs' buffer and the perfusion pressure recorded. The renal perfusion pressure was raised similarly (120-180 mmHg) in the control and chronic L-NAME isolated perfused kidneys (IPK) with phenylephrine (PE;5-20μM) and vasodilatations induced by Ach (1nmol) and SNP (3nmol) were recorded (n=5-7). Rings of RTA were taken from the same animals and mounted in organ baths. They were constricted to approx. 1.25 g tension by phenylephrine (0.1-0.3μM) and relaxations to ACh (0.01-1μM) or SNP (0.1-30nM) were measured (n=5-7).

Mean arterial blood pressure was elevated in rats treated with chronic L-NAME (chronic L-NAME, 153.1±10.6mmHg; control, 114.2±4.8mmHg). The depressor response to ACh was increased in the chronic L-NAME treated rats (chronic L-NAME, 48.7±4.4mmHg; control, 31.1±2.3mmHg) as was that to SNP (chronic L-NAME, 83.4±10mmHg; control, 40±4.9mmHg). The vasodilatations induced by ACh in the IPK were unchanged in tissues from chronic L-NAME treated animals (chronic L-NAME, 79.6±9.4 mmHg; control, 74.4±10.1mmHg) as were those to SNP (chronic L-NAME, 65.8±16mmHg; control, 46.7±10.3mmHg). In the IPK, acute administration of L-NAME (0.1-100μM, n=4-5) caused a concentration dependent inhibition of the vasodilatations induced by ACh (e.g. 100μM L-NAME inhibited the depressor response to ACh by 69±6%), whereas those to SNP were unchanged. Relaxations of the RTA (with endothelium intact) induced by ACh were absent in tissues taken from the chronic L-NAME treated rats (e.g. relaxation to 1μM ACh, control, 88.5±5.4%; chronic L-NAME, 18.3±15%). In control tissues, acute L-NAME produced a dose dependent inhibition of the relaxations of the RTA induced by ACh that was greater than that seen in the IPK (e.g. relaxation to ACh (1μM), 100μM L-NAME, -2.4±0.8%; control, 80.7±4%). The relaxations of the RTA to SNP were unaltered by chronic L-NAME treatment.

Thus chronic administration of L-NAME to rats elevates arterial blood pressure and attenuates the ACh induced vascular relaxation in the RTA. However, chronic L-NAME has no effect on the vasodilations to ACh or SNP in the IPK and enhances the ACh response *in vivo*. Acute administration of L-NAME abolishes the ACh response in the RTA, but is less effective in the IPK. Thus, when orally and chronically administered the plasma levels of L-NAME achieved may well be sufficent to block NOS completely in the RTA, but not in the kidney.

This work was supported by Glaxo Group Research Ltd. and CEB is a Wellcome Veterinary Fellow.
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445P AN INVESTIGATION OF THE INTERACTION BETWEEN L-NAME AND ANGIOTENSIN II ON BLOOD PRESSURE AND RENAL SYMPATHETIC NERVE ACTIVITY IN α-CHLORALOSE ANAESTHETIZED RATS

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Central inhibition of nitric oxide (NO) synthesis increases renal nerve activity and blood pressure (Togashi *et al.*, 1992). This effect of NO synthesis inhibition has been demonstrated to play an important role in the hypertension observed in rats in which NO synthesis has been chronically inhibited (Cunha *et al.*, 1993). Furthermore, this hypertension has also been shown to be attenuated by pretreatment with captopril (de Moura *et al.*, 1992). These observations suggest that the hypertension induced by NO synthesis inhibition may involve changes in renal nerve activity and angiotensin II levels. Therefore, initial experiments were carried out to investigate the effects of the ACE inhibitor, captopril and the AT_1 receptor antagonist losartan on the acute hypertension and changes in renal nerve activity induced by L-NAME in anaesthetized rats.

Arterial blood pressure (BP) and heart rate (HR) were measured from the femoral artery in spontaneously breathing male Wistar rats (250- 390g) in which anaesthesia had been induced with halothane and maintained with α-chloralose (80 mg kg^{-1}). The renal nerve activity (RNA) was measured and quantified as previously described (Anderson *et al.*, 1992). After a 5-10 min stabilization period an injection of L-NAME (10mg kg^{-1}) via the femoral vein was followed after 10 min by either captopril (1 mg kg^{-1}) or losartan (3 mg kg^{-1}) and this was repeated 10 min later.

In the captopril group (n=8), L-NAME after 10 min had caused an increase in BP of 47 ± 6 mmHg (mean ± s.e. mean) which was associated with decreases in heart rate of 31 ± 9 beats min^{-1} and in RNA of 73 ± 6 % from baseline values. Captopril reduced this increase in BP caused by L-NAME to 27 ± 4 mmHg above baseline while HR and RNA returned to near baseline values, +9 ± 6 beats min^{-1} and +2 ± 5 % by 10 min. In the losartan group (n=6), L-NAME again caused an increase in BP of 56 ± 6 mmHg and decreases in HR of 42 ± 14 beats min^{-1} and in RNA of 86 ± 6%. Losartan reduced the L-NAME induced rise in BP to 24 ± 4 mmHg while HR returned to near baseline, +12 ± 9 beats min^{-1}, and RNA +30 ± 11 %. The second dose of captopril or losartan had only a small effect on these changes.

The elevated BP after captopril or losartan would be expected to evoke a baroreceptor mediated sympathoinhibition and a bradycardia. However, both captopril and losartan returned RNA and HR to near baseline values. This indicates that these drugs are having a central action, presumably by interfering with the actions of angiotensin II, to reduce the sympathoinhibition and bradycardia caused by the rise in BP induced by L-NAME.

We wish to thank the British Council & CAPES (Brazil) for support and Mr. M.A. Dantas and Dr M.P.Gilbey for assistance.

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446P EFFECTS OF PROSTAGLANDINS ON STIMULATED RELEASE OF NITRIC OXIDE BY ANGIOTENSIN II FROM HUMAN SAPHENOUS VEIN

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Angiotensin II (A II) causes contraction of the human saphenous vein (HSV) and stimulates release of prostacyclin (PGI_2) from this tissue (Barker et al. 1993). This output of PGI_2 (as 6-oxo-$PGF_{1\alpha}$) was further increased (7-fold) by inhibition of nitric oxide (NO) synthesis with L-NAME (200 μM) suggesting an inhibitory role for NO in PGI_2 release (Barker et al. 1994). In the present series of experiments we have explored the possibility of an inhibitory effect of prostaglandins (PGs) on NO synthesis, in samples of HSV obtained from patients undergoing coronary artery bypass surgery.
Relaxation of smooth muscle induced by NO is mediated by cGMP and we have measured cGMP levels as an indication of NO synthesis in HSV (Schini et al. 1989). Rings of HSV (2-4mm wide) were mounted in organ baths in Krebs Henseleit buffer (KH). Only those rings that relaxed to bradykinin (10 μM) after precontraction with phenylephrine (10 μM) were used in this study; Several rings could be cut from each sample of HSV (one sample only was obtained from each patient) and these matched rings were used under four experimental conditions; one group had no pre-treatment; the others were treated with the cyclo-oxygenase inhibitor piroxicam (PC, 5 μM) or PC and iloprost (a stable PGI_2 analogue; 2.5 ng ml^{-1}) or PC and L-NAME (200 μM) for 15 min; all groups were then stimulated with A II (100 nM). Samples of KH bathing the rings were removed just before (t=0), and then at 1 and 3 min after the addition of A II. The cGMP content of the buffer samples was measured by radioimmunoassay. Results given are the mean (± se mean) values as fmol cGMP mg of wet $weight^{-1}$ from at least 3 samples. Means were compared using Students t-test, significance was assumed when $P<0.05$. On A II stimulation, untreated rings released 3.3 ± 0.5 fmol mg^{-1} at 1 min but this was not increased above release at t=0 (2.1 ± 0.6 fmol mg^{-1}; n=5). Release of cGMP from PC treated rings was higher at 1 and 3 min, reaching a maximum of 6.7 ± 0.9 fmol mg^{-1} (n=8) at 3 min. This enhanced release was abolished by co-treatment with iloprost and PC giving values at 3 min of 2.6 ± 1.3 fmol mg^{-1} (n=3) which were not different from those under basal unstimulated conditions and less than from PC treated rings. Finally co-treatment with L-NAME and PC also prevented the stimulated increase in cGMP levels (4.1 ± 1.0 fmol mg^{-1} at 3 min; n=4).
From these results we conclude that PGI_2 generated in the HSV could inhibit NO output stimulated by A II. This finding along with the previous result that NO can inhibit A II stimulated PGI_2 release from HSV suggests the presence of a mechanism in HSV whereby NO and PGI_2 regulate each others synthesis.

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447P ATTENUATION OF CYTOKINE-STIMULATED NITRIC OXIDE PRODUCTION BY h-ANP IN HUMAN RENAL PROXIMAL TUBULAR CELLS IS NOT MEDIATED BY CYCLIC GMP

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We have demonstrated h-ANP receptor function in primary cultures of human proximal tubular (HPT) cells by the production of cyclic GMP in the presence of h-ANP (Chatterjee et al.,1993). HPT cells can also be stimulated to produce nitric oxide (NO) by the induction of nitric oxide synthetase when stimulated by a combination of the cytokines IL-1β, TNF-α and IFN-γ. The recent evidence that both h-ANP and NO may possess immunomodulatory functions prompted us to examine the effect of h-ANP upon cytokine-induced NO production in HPT cells.

HPT cells were isolated from human nephrectomy samples. $1x10^5$ cells were plated on to 24 well plates and allowed to reach confluence. Cells were incubated with h-ANP (10^{-10}M - 10^{-6}M), a cytokine "cocktail" containing IL-1β (10u/ml), TNF-α (10ng/ml) and IFN-γ (100u/ml) or with a combination of h-ANP and cytokines. HPT cells were incubated with h-ANP for periods up to 24 hours, followed by cytokine stimulation for 24 hours, or were incubated with both h-ANP and cytokines for periods up to 24 hours. Supernatant was removed for estimation of NO (Griess) and cyclic GMP.

A dose-dependent increase in cyclic GMP production was seen (from 32.1 ± 8.6 baseline to 76.2 ± 9.2, 43.4 ± 4.1, 35.5 ± 7.0 pmol/mg protein/24 hours, mean ± s.d., n = 3) when the cells were incubated with 10^{-6}M, 10^{-8}M and 10^{-10}M h-ANP respectively. Incubation for 24 hours with the cytokines produced a significant increase in both NO (20 fold) and cyclic GMP production (1149.6 ± 186.3 pmol/mg protein/24 hours, $p<0.05$, n = 3). The increase in cyclic GMP and NO production was attenuated when cells were preincubated with h-ANP. Preincubation with 10^{-6}M h-ANP inhibited NO production by 26.7%, 24.2%, 47.6% and 61.3% ($p<0.05$) at 1,4,12,24 hours respectively (n = 3). Coincubation with 10^{-6}M h-ANP also resulted in a significant but less marked effect. Incubation with dibromo cyclic GMP for equivalent times did not affect the cytokine-stimulated NO production, indicating that these observations are independent of the activation of particulate guanylate cyclase by h-ANP.

These results clearly show that h-ANP significantly inhibits cytokine-stimulated production of NO from HPT cells and suggest that h-ANP may possess an immunomodulatory role independent of classical ANP-A or B receptor function.

Chatterjee, P.K. et al (1993) J.Am.Soc.Nephrol. 4:444P

448P INVOLVEMENT AND CELLULAR SOURCE OF INDUCED NITRIC OXIDE SYNTHASE IN ZYMOSAN PERITONITIS IN THE RAT

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Excessive nitric oxide (NO) production following NO synthase (NOS) induction has been implicated in the vascular leakage produced in the rat colon after endotoxin (Boughton - Smith et al, 1993) and in carrageenin paw oedema (Ialenti et al, 1992). The cellular source of the NO that increases vascular permeability could come from the vasculature or from infiltrating or resident leucocytes. In the present study the involvement of NOS induction in zymosan peritonitis was investigated and the contribution of infiltrating leucocytes to changes in NO levels and vascular permeability determined.

Peritonitis was produced in Wistar rats (male, 250 - 330 g) by zymosan administration, 10 mg in 1ml phosphate buffered saline (PBS) i.p. Cell counts were conducted on peritoneal lavage (4 ml, heparinised saline, 100 U ml^{-1}) after 2 h, 4 h and 6 h, and vascular permeability determined as the leakage of Pontamine sky blue (2.5%, 0.5 ml, i.v.) measured spectrophotometrically (610 nm). NO levels in peritoneal lavage were determined by the Griess assay, and NO synthase activity in lavage cells determined as the conversion of [^{14}C]-L-arginine to L-citrulline (Boughton - Smith et al, 1993). Time dependent increases in plasma leakage (161 $\pm$ 66 % increase at 6 h, mean $\pm$ s.e. mean, n = 5, $P < 0.05$) and cell infiltration (12 $\pm$ 2 x 10^6 cells ml^{-1} at 6 h, n = 6, $P < 0.01$) after zymosan were accompanied by increases in NO levels of 14 $\pm$ 2 μM and 21 $\pm$ 3 μM after 4 h and 6 h respectively (n = 6, $P < 0.05$), and induction of calcium-independent NOS activity in lavage cells of 8 $\pm$ 1 and 13 $\pm$ 3 pmol min^{-1} 10^6 cells, at 4 h and 6 h respectively (n = 5, $P < 0.01$). Colchicine (3 mg kg^{-1}, s.c.), 5 min before zymosan, abolished cellular infiltration (n = 5, $P < 0.01$,), reduced plasma leakage (46 $\pm$ 25 % inhibition) to a level no longer different from control, but had no effect on increases in NO. Dexamethasone (3 mg kg^{-1} s.c. -2 h) abolished increases in NO (n = 5, $P < 0.01$) and reduced plasma leakage (67 $\pm$ 13 % inhibition, $P < 0.05$). L-NAME (30 mg kg^{-1}, i.p.), 2 h after zymosan, abolished increases in NO (n = 10, $P < 0.01$) and reduced plasma leakage (62 $\pm$ 16 % inhibition, n = 5, $P < 0.01$).

The results show that induction of NOS activity occurs in zymosan peritonitis in the rat and suggest that NO plays a role in the vascular leakage. The predominant cellullar source of NOS activity in zymosan peritonitis is not from infiltrating leucocytes but resident cells, of which both vascular cells and leucocytes could contribute.

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449P PROTECTIVE ACTIONS OF THE NITRIC OXIDE DONOR S-NITROSO-GLUTATHIONE OR AN ANTI-NEUTROPHIL ANTIBODY IN ACUTE MICROVASCULAR INJURY IN RAT INTESTINE

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Inhibition of constitutive nitric oxide (NO) synthase augments acute intestinal vascular injury provoked by high dose endotoxin challenge, an effect attenuated by concurrent infusion of a nitrovasodilator (Boughton-Smith et al, 1990). Neutrophil adhesion to the endothelium, which is considered an initial event in intestinal microvascular injury, is attenuated by exogenous NO (Kubes et al, 1991). We have, therefore, now compared the effects of the NO donor, S-nitroso-glutathione (SNOG) with those of pretreatment with an anti-neutrophil antibody, on the acute microvascular injury in the intestine induced by the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) following challenge with lower doses of endotoxin (Laszlo et al, 1993).

E. coli lipopolysaccharide (LPS; 3mg kg^{-1}, i.v.) was administered to male rats under pentobarbitone-anaesthesia, accompanied by the administration of L-NAME (5 mg kg^{-1}, s.c.). [^{125}I] human serum albumin (2μCi kg^{-1}, i.v.) was administered concurrently for the subsequent determination of vascular albumin leakage, as an index of microvascular injury, 1h after challenge. Following LPS administration, L-NAME provoked albumin leakage in the ileum and colon (by Δ473±216 and 329±45μl plasma g^{-1} tissue, respectively; mean±s.e.mean, n=5; $P<0.05$ for both by ANOVA) and elevated systemic arterial blood pressure (BP) by Δ43±5mmHg after 1h. In a control study, infusion of phenylephrine (10μg kg^{-1} min^{-1} i.v. for 1h) likewise elevated BP by Δ41±6 mmHg (n=4), but did not augment LPS-induced intestinal vascular leakage after 1h. Intravenous infusion of SNOG (1-10 μg kg^{-1} min^{-1} for 1h) dose-dependently attenuated the intestinal microvascular leakage induced by L-NAME and LPS. Thus, SNOG (1μg kg^{-1} min^{-1} i.v. for 1h) reduced this albumin leakage by 36±11% and 45±10% (n=5, $P<0.05$) in ileum and colon, but did not significantly affect the BP responses to L-NAME and LPS; SNOG (10 μg kg^{-1} min^{-1}, i.v.) abolished ileal and colonic microvascular leakage (n=5; $P<0.01$) and returned the elevated BP to resting values. Pretreatment of rats with rat anti-neutrophil serum (Accurate Chemicals, New York; 0.4ml kg^{-1}, i.p.), which reduced the number of neutrophils in the systemic blood by 85±5% (n=5), inhibited the microvascular leakage provoked by LPS and L-NAME by 49±12% and 43±15% (n=5; $P<0.01$) in the ileum and colon, respectively.

These findings demonstrate that the NO donor, SNOG, can attenuate microvascular injury provoked by L-NAME in LPS-challenged rats, in doses that do not exert significant actions on BP. This could support a protective effect of NO on the microvasculature, in addition to any local vasodilator action, such as through attenuation of neutrophil adherence to the endothelium. Indeed, the protective actions of the anti-neutrophil antibody implicate involvement of these cells, along with the release of neutrophil-derived pro-inflammatory mediators such as Paf, thromboxanes or oxygen-derived free radicals, in this model of acute microvascular injury in the rat intestine.

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450P THE EFFECT OF CHRONIC INGESTION OF A NITRIC OXIDE SYNTHASE INHIBITOR ON CARDIAC TISSUE ENERGY METABOLISM

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Chronic oral administration of the nitric oxide synthase inhibitor, N^G-nitro-*L*-arginine-methyl ester (*L*-NAME), has been shown to produce sustained hypertension (Gardiner *et al.*, 1992). The aim of the present experiment was to determine the effect of chronic ingestion of *L*-NAME on cardiac tissue energy metabolism. Fourteen male, homozygous Brattleboro rats (350-450 g) drank either water (control; n=7) or *L*-NAME (n=7; 0.05 mg ml^{-1} in drinking water) *ad libitum* for 4 weeks. At the end of this period, the chest wall was opened under anaesthesia (sodium methohexitone 60 mg kg^{-1} i.p.) and the heart was rapidly removed and snap frozen in liquid nitrogen. Subsequently, all hearts were freeze dried and ventricular adenosine triphosphate (ATP), phosphocreatine (PCr), free creatine (Cr) and lactate concentrations were measured (Harris *et al.*, 1974).

Table 1. Energy metabolite concentrations (mmol kg^{-1} dry tissue) in hearts from control (n=7) and *L*-NAME treated rats (n=7). Values are mean ± s.e. mean.

	ATP	PCr	Cr	Total Cr (ΣPCr+Cr)	Lactate
Control	20.50±0.68	3.77±0.56	65.24±1.56	69.01±1.66	26.57±2.43
L-NAME	16.21±0.39***	1.94±0.24**	57.06±1.88*	59.00±1.90**	38.10±1.42**

* P<0.05, ** P<0.01, *** P<0.001; Control versus *L*-NAME (unpaired Student's t-test).

The greater lactate production and PCr degradation in the hearts from *L*-NAME treated animals indicates that anaerobic energy production was greater in these hearts, suggesting the energy demand of contraction was greater and/or that oxygen delivery or extraction was lower compared with controls. The lower ATP concentration in the hearts from *L*-NAME treated animals also indicates that adenine nucleotide loss was greater in those tissues, and suggests that the relative degree of metabolic stress was greater.
The reduction in total Cr concentration following *L*-NAME administration was unexpected, but suggests *L*-NAME was influencing Cr biosynthesis in the liver, and/or Cr uptake by the myocardium.

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451P EFFECT OF N^G-MONOMETHYL-L-ARGININE ON VASODILATOR RESPONSES TO BRADYKININ AND SUBSTANCE P IN THE HUMAN FOREARM VASCULAR BED

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A substantial fraction of the response of human forearm vasculature to muscarinic agonists is mediated through pathways that are independent of L-arginine/nitric oxide (NO) (Chowienczyk *et al.*, 1993), so there is a need for agonists with greater dependence on this pathway to facilitate investigation of endothelial function in this vascular bed. We have therefore examined the contribution of the L-arginine/NO pathway to the vasodilation produced by bradykinin (BK) and substance P (SP) in the human forearm vascular bed. Sixteen healthy normotensive men (21-49 yrs) were studied, each on 2 separate occasions. The protocol was approved by Lewisham and North Southwark ethics committee. Subjects were studied resting supine in a temperature controlled laboratory. Drugs were infused via a 27 gauge steel cannula into the left brachial artery and blood flow in both forearms (FBF) measured by venous occlusion plethysmography using mercury in silastic strain gauges. Eight subjects received BK (100 pmol min^{-1}) on each occasion and a further eight received SP (1 pmol min^{-1}). Subjects received saline for 12 min followed by an 18 min infusion of vasodilator peptide. On one occasion N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthase was co-infused at doses of 2 and 4 μmols min^{-1} during the final 12 min of the peptide infusion, each dose for 6 min. On the other occasion saline vehicle was co-infused instead of L-NMMA.

FBF in the control arm did not change significantly during the experiment on any study day. Before co-infusion of L-NMMA BK increased FBF from 3.1 ± 0.2 to 9.4 ± 1.0 ml min^{-1} $100ml^{-1}$ on the saline day and from 2.8 ± 0.2 to 9.3 ± 1.0 ml min^{-1} $100ml^{-1}$ on the L-NMMA day; SP increased FBF from 3.0 ± 0.2 to 5.6 ± 0.5 ml min^{-1} $100ml^{-1}$ on the saline day and from 3.4 ± 0.2 to 6.3 ± 0.7 ml min^{-1} $100ml^{-1}$ on the L-NMMA day. Co-infusion of L-NMMA inhibited response to BK relative to saline control, by 9% ± 14% (NS) and 42% ± 14% ($P < 0.001$) at doses of 2 and 4 μmol min^{-1} respectively. Response to SP relative to saline was also inhibited by L-NMMA, by 27% ± 8% at a dose of 2 μmol min^{-1} ($P < 0.01$) and 70% ± 13% at a dose of 4 μmol min^{-1} ($P < 0.001$). SP may be a useful agonist with which to study abnormalities of endothelial function in this vascular bed.

Chowienczyk, P.J., Cockcroft, J.R. & Ritter, J.M. (1993) Br. J. Pharmac. **110**, 736-738

452P NITRIC OXIDE INDEPENDENT VASODILATION BY ACETYLCHOLINE IN HUMAN FOREARM VASCULATURE IS SELECTIVELY POTENTIATED BY EDROPHONIUM

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N^G-monomethyl-L-arginine (L-NMMA, a nitric oxide synthase inhibitor) inhibits vasodilator responses to acetylcholine but not methacholine in human forearm vasculature (Chowienczyk *et al.*, 1993). To investigate whether this difference results from the relative susceptibility of these agonists to hydrolysis by acetylcholinesterase we studied effects of L-NMMA on vasodilator responses to brachial artery administration of acetylcholine alone and in the presence of the acetylcholinesterase inhibitor edrophonium. The study was approved by the West Lambeth Health Authority Ethics Committee. Eight healthy volunteers were studied on 2 occasions. On both occasions they received 2 stepwise brachial artery infusions of 3 cumulative doses of acetylcholine, each dose for 4 min. The two infusions were separated by a 20 min infusion of saline. Five min before the second acetylcholine infusion L-NMMA (4 μmol min^{-1}) infusion was commenced and continued throughout the second infusion of acetylcholine. On one occasion acetylcholine alone (16, 55 and 165 nmol min^{-1}) was used and on the other acetylcholine (1.6, 5.5 and 16 nmol min^{-1}) was co-infused with edrophonium (0.5 μmol min^{-1}). Edrophonium infusion was commenced 6 min before the start of each acetylcholine infusion and stopped after each acetylcholine infusion. Forearm blood flow (ml min^{-1} per 100 ml forearm volume) was measured using venous occlusion strain gauge plethysmography. Acetylcholine alone (16, 55 and 165 nmol min^{-1}) increased blood flow from 2.9 ± 0.4 (mean ± s.e.mean) to 5.3 ± 1.0, 10.4 ± 2.0 and 16.4 ± 2.7 ml min^{-1} 100 ml^{-1} respectively. Edrophonium alone had no effect on forearm blood flow (1.82 ± 0.4 and 1.80 ± 0.4 ml min^{-1} 100 ml^{-1} before and after edrophonium respectively). Edrophonium potentiated responses to acetylcholine so that acetylcholine (1.6, 5.5 and 16 nmol min^{-1}) with edrophonium produced similar vasodilation to acetylcholine alone (16, 55 and 165 nmol min^{-1}) increasing blood flow from 1.8 ± 0.4 to 7.4 ± 1.4, 10.5 ± 1.3 and 13.6 ± 2.0 ml min^{-1} 100 ml^{-1} respectively. Inhibition of vasodilation by L-NMMA was significantly greater for acetylcholine alone than for acetylcholine with edrophonium ($P<0.01$), ranging from 64 ± 19% to 33 ± 11% for acetylcholine alone (lowest to highest dose) and from 31 ± 9% to -2 ± 13% for acetylcholine with edrophonium. These results suggest that actions of acetylcholine in human forearm resistance vessels are mediated both through the L-arginine/NO pathway and through an L-NMMA resistant pathway. The L-NMMA resistant pathway is selectively potentiated by edrophonium.

Chowienczyk, P.J., Cockcroft, J.R. & Ritter, J.M. (1993) Br. J. Pharmacol. **110,** 736-738.

453P CALCITONIN GENE-RELATED PEPTIDE (CGRP) AS AN ENDOGENOUS VASODILATOR: *IN VIVO* IMMUNOBLOCKADE STUDIES WITH AN ANTI-CGRP MONOCLONAL ANTIBODY AND ITS FAB' FRAGMENT

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Immunoblockade is being increasingly used as a pharmacological technique for probing the physiological role of neuropeptides (van Oers *et al.*, 1992). However, it remains to be clearly established as an effective experimental technique *in vivo*. We investigated immunoblockade as an *in vivo* technique for studying the vasodilator role of the calcitonin gene-related peptide (CGRP). The results of immunoblockade were compared to those obtained by receptor blockade with the C-terminal 8-37 fragment of human αCGRP ($H\alpha CGRP_{8-37}$).

An anti-CGRP monoclonal antibody (MAb; coded C4.19) IgG and its Fab' fragment were used for immunoblockade. The effects of MAb C4.19 and $H\alpha CGRP_{8-37}$ on the hypotensive responses to exogenous rat αCGRP ($R\alpha CGRP$) and substance P were investigated in the pentobarbitone-anaesthetized rat (60 mg kg^{-1} i.p.). The role of CGRP in mediating antidromic vasodilation was investigated by blockade of the increase in hind paw skin blood flow induced by saphenous nerve stimulation in the pentobarbitone-anaesthetized rat. The saphenous nerve was stimulated (2 Hz, 10 V, 1 ms, for 10 s) after treatment with vehicle and again after an interval of 30 min or longer following treatment with antibody preparations or $H\alpha CGRP_{8-37}$. Change in skin blood flow was measured by laser Doppler fluxmetry. The maximum change in flux (Fmax) and area under the flux-time curve (AUC) attributable to nerve stimulation were calculated. All drugs were given intravenously. The paired and unpaired t tests were used for within- and between-animal comparisons respectively. $P < 0.05$ was considered statistically significant.

MAb C4.19 IgG (1mg) and its Fab' fragment (2mg) abolished the hypotensive response to 0.1 nmol kg^{-1} $R\alpha CGRP$ but had no significant effect on the hypotensive response to 0.1 nmol kg^{-1} substance P. The dose-hypotensive response relationship of $R\alpha CGRP$ was shifted to the right by the IgG and Fab' fragment. $H\alpha CGRP_{8-37}$ (100 nmol kg^{-1}) also blocked the hypotensive effect of 0.1 nmol kg^{-1} $R\alpha CGRP$ significantly. MAb C4.19 IgG at doses up to 3mg given up to 2 h prior to nerve stimulation did not block the skin blood flow response to antidromic stimulation of the saphenous nerve. In contrast, Fmax and AUC were significantly blocked 30 min following administration of the Fab' fragment (2mg). The mean percentage decrease in Fmax and AUC were 28.6% and 62.9% respectively. Normal mouse Fab' fragment (2mg) had no significant effect on Fmax and AUC compared with vehicle control. $H\alpha CGRP_{8-37}$ (100 nmol kg^{-1}) given 3 min prior to nerve stimulation significantly decreased Fmax and AUC by 20.9% and 74.6% respectively. The mean percentage changes in skin blood flow parameters due to MAb C4.19 Fab' fragment were significantly different from those due to normal mouse Fab' fragment but not from those due to $H\alpha CGRP_{8-37}$. The results show that immunoblockade with an anti-CGRP MAb may be used to demonstrate the physiological role of endogenous CGRP. However, Fab' fragments are required for effective distribution to the site of action. Our results agree with those of Escott & Brain (1993) who also found that $H\alpha CGRP_{8-37}$ blocked antidromic vasodilation, and further support the role of CGRP in mediating skin vasodilation.

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454P EFFECTS OF A XANTHINE OXIDASE/HYPOXANTHINE FREE RADICAL/REACTIVE OXYGEN SPECIES ON VASCULAR FUNCTION IN RABBIT AORTAE

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Free radicals (FR) and reactive oxygen species (ROS) have been implicated as one of the major factors involved in ischaemia reperfusion injury (Halliwell, 1989). A previous study demonstrated that a xanthine oxidase / hypoxanthine (XO/HX) FR/ROS generating system impairs carbachol induced relaxation in rings of thoracic aorta from New Zealand White rabbits. Furthermore, that this impairment of response could be mimicked by H_2O_2 (Dowell, 1992). The aim of this study was to investigate the effects of the same XO/HX system on the vascular reactivity of the smooth muscle in rabbit aortic rings. XO (20mU/ml), HX (250μM), $FeCl_3$ (25μM) and EDTA (37.5μM) were used to generate $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$. New Zealand White rabbit aortic rings (2mm) were suspended in an organ bath filled with Krebs bicarbonate buffer at 37°C, aerated with 95% O_2 and 5% CO_2. Control cumulative concentration response curves were obtained to either phenylephrine (PE) (10^{-8}M-10^{-5}M); or sodium nitroprusside (SNP) (10^{-8}M-10^{-5}M) following preconstriction with the ED_{50} concentration of PE. After wash out and re-equilibration rings were exposed to either the XO/HX system, H_2O_2 or vehicle for 30 minutes. Baths were washed and the cumulative concentration response curves to PE or SNP were repeated. Responses to PE were expressed in grams tension / mg tissue. Responses to SNP were expressed as a percentage of the phenylephrine induced contraction. Values are given as mean ± SD. Comparisons were made using ANOVA ($p<0.05$).

Exposure to vehicle (30 mins) resulted in a small, significant decrease in response to PE at $3x10^{-8}$M, and a small non-significant decrease in response to SNP. Thus in all further analysis, comparisons were made with data from vehicle exposed rings. Exposure to the XO/HX system resulted in an impaired contractile response to PE (Maximum response 0.79 ± 0.11 gT/mg tissue in the vehicle control, 0.70 ± 0.11 gT/mg tissue following XO/HX exposure, n=12). Direct addition of 500μM H_2O_2 lead to a similar impairment of response (0.91 ± 0.12 gT/mg tissue in the vehicle control, 0.83 ± 0.09 gT/mg tissue following H_2O_2 exposure, n=6). SNP induced relaxation following XO/HX exposure was also found to be impaired. Responses from 10^{-8}M to 10^{-6}M were significantly reduced (10^{-7}M SNP induced 64 ± 14% relaxation in vehicle control and 48 ± 8% relaxation following XO/HX exposure, n=6). As with the PE responses, it was found that these effects could be mimicked by 500μM H_2O_2 (10^{-7}M SNP induced 75 ± 13% relaxation in vehicle control and 45 ± 16% relaxation following H_2O_2 exposure, n=9).

These data suggest that for vascular smooth muscle, as for endothelium, the primary ROS responsible for impairing vascular reactivity following exposure to the XO/HX FR/ROS generating system is H_2O_2.

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455P ROLE OF ANTIOXIDANTS IN THE H_2O_2 INHIBITION OF HISTAMINE RELEASE FROM RODENT MAST CELLS

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We have previously shown in rat peritoneal mast cells (RPMC) that H_2O_2 produces a marked and dose-related inhibition of histamine release (HR) induced by compound 48/80, A23187, NaF or anti-IgE (Moulson et al., 1994). In view of the known heterogeneity of mast cells, it was of interest to compare the action of H_2O_2 on stimulated HR from RPMC and tissue mast cells from rat mesentery and uterus (RMMC and RUMC). The effects of antioxidant enzymes, catalase, glutathione peroxidase and superoxide dismutase (SOD) on the action of H_2O_2 were also studied (Harris et al., 1992).

RPMC were obtained by direct lavage. RMMC and RUMC were obtained by collagenase digestion (120 u/ml, 60 min). For anti-IgE experiments, the animals were sensitized with *Nippostrongylus brasiliensis*. Catalase activity was monitored by disappearance of H_2O_2 at 240 nm. Measuring the decrease in NADPH concentration at 340 nm was used to determine total cellular glutathione peroxidase activity. Trypan blue exclusion was used to assess cell viability.

	RPMC		RUMC		RMMC	
H_2O_2 (mM)	0.1	1.0	0.1	1.0	0.1	1.0
Anti-IgE (1/300 dilution)	92.5±0.9	97.8±1.3	8.7±2.5	12.2±4.2	1.6±2.7	4.5±4.0
48/80 (0.5 μg/ml)	87.9±1.9	96.4±2.0	11.2±6.7	13.5±7.6	-3.0±1.1	-4.5±1.3
NaF (10 mM)	91.3±2.2	99.3±1.7	17.2±3.7	20.1±5.9	-3.6±1.8	-3.0±1.7

The table shows percentage inhibition (mean±s.e.m., N=4) of HR by H_2O_2. Cells were preincubated with H_2O_2 for 30 min and then for a further 10-30 min following stimulation. HR for all stimulants was 36-58%.

H_2O_2 produced little or no inhibition of HR with all stimulants in RMMC and RUMC in contrast to the marked inhibition observed in RPMC. For all cell types, the cell viability was not changed by treatment with H_2O_2. RMMC and RUMC caused a decrease of 23.1±3.4% and 16.6±4.8% respectively in H_2O_2 absorption compared to controls. This decrease was only 8.7±4.7% with RPMC. No glutathione peroxidase activity was observed in RPMC and RUMC. With RMMC, a glutathione peroxidase activity of 0.015 units per 10^6 cells was seen. Preincubating cells with SOD (20 μg/ml, 10 min), then H_2O_2 (30 min), followed by stimulant (10-30 min), had no effect on the inhibition of HR by H_2O_2. In contrast, catalase (10 u/ml, 10 min), completely reversed the inhibitory effects of H_2O_2 for all three stimulants. These data suggest that H_2O_2 has a selective action on inhibiting HR from RPMC, but has little or no effect on RUMC and RMMC possibly due to higher intracellular concentrations of the H_2O_2 scavengers, catalase and glutathione peroxidase in the latter cells.

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456P ENDOGENOUS EXPRESSION OF HISTAMINE H_1-RECEPTORS FUNCTIONALLY COUPLED TO PHOSPHOINOSITIDE HYDROLYSIS IN C6 GLIOMA CELLS: REGULATION BY CYCLIC AMP

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The presence of histamine H_1 receptors coupled to phospholipase C, has been shown both in primary astrocytes derived from rat brain (Kondou *et al.*, 1991) and in human astrocytoma cells (Nakahata & Harden, 1987). C6 glioma cells, however, have recently been transfected with isolated genes for the rat (Fujimoto *et al.*, 1993) and bovine (Liu et al., 1992) histamine H_1 receptors. Here we demonstrate the presence of endogenous histamine H_1 receptors functionally linked to phosphoinositide turnover in C6 glioma cells and the modulation of this response by agents which stimulate the production of cyclic AMP.

Confluent monolayer cultures of C6 cells were loaded for 24 hours with [^{3}H]*myo*-inositol (37KBq/well) in inositol-free DMEM. Inositol phospholipid hydrolysis in prelabelled cells was investigated using the method previously described by White *et al.* (1993). Cells were exposed to agonists and antagonists in the presence of 20mM lithium chloride and total [^{3}H]inositol phosphates were isolated by anion exchange chromatography.

Histamine (1mM, 2.8 ± 0.9 fold over basal levels, $p<0.01$, $EC_{50}=24.9 \pm 5.1\mu M$, n=7) and the H_1 receptor-selective agonist, 2-thiazolylethylamine, (1mM, 1.9 ± 0.2 fold over basal levels, $p<0.01$, $EC_{50}=90.6 \pm 18.3\mu M$, n=3) caused significant, concentration-dependent accumulations of total [^{3}H]inositol phosphates after 10 minutes incubation with C6 glioma cells. The response to 0.1mM histamine (10 minute stimulation) was antagonized by low concentrations of mepyramine (apparent $K_D=1.0 \pm 0.2$nM, n=3) and (+)-chlorpheniramine (apparent $K_D=4.4 \pm 0.6$nM, n=4). However, pre-treatment with either tiotidine, at concentrations up to 10μM (n=3), or thioperamide, at concentrations up to 1μM (n=3) did not significantly affect histamine-stimulated inositol phosphate accumulation. Incubation of the cells with forskolin (10μM, 37.2 ± 4.3% inhibition, $p<0.01$, n=6) or isoprenaline (1μM, 42.7 ± 12.4% inhibition, $p<0.01$, n=3) for 10 minutes prior to the addition of 0.1mM histamine (for a further 10 minutes) significantly inhibited histamine-stimulated (0.1mM) total [^{3}H]inositol phosphates accumulation. In contrast, 1,9-dideoxyforskolin, at concentrations up to 10μM, caused no significant alteration of the histamine-mediated phosphoinositol response ($p>0.05$, n=3).

These data indicate the presence of endogenous histamine H_1-receptors in C6 glioma cells and suggest that elevation of cyclic AMP levels can modify the phosphoinositide response elicited on their activation.

MCP holds a Wellcome Trust Prize Studentship.

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457P KINETIC ANALYSIS OF MUSCARINIC ACh RECEPTOR TRAFFICKING IN NG108-15 CELLS

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We have shown (Koenig & Edwardson, 1994) that in unstimulated NG108-15 cells, muscarinic ACh receptors arrive at the plasma membrane almost exclusively along the 'synthetic' route. In agonist-stimulated cells, receptors are internalized into endosomes, from which they may either be recycled to the plasma membrane or degraded in lysosomes. In the present study we have developed a kinetic model of receptor trafficking that includes four transport steps - Golgi to plasma membrane (linear), endosome to plasma membrane (exponential), plasma membrane to endosome (exponential) and endosome to lysosome (linear) - governed by rate constants k_1-k_4, respectively. We have derived a general equation that describes the changes in receptor number at the plasma membrane in response to agonist stimulation. By following receptor trafficking under various conditions, we have determined k_1-k_4, and also the size of the endosomal receptor pool created by agonist stimulation.

Receptor trafficking was monitored through changes in receptor number at the plasma membrane. Surface receptors were quantitated through the binding of the membrane-impermeant radioligand [^{3}H]N-methylscopolamine ([^{3}H]NMS). Delivery of receptors to the plasma membrane was detected through the recovery of [^{3}H]NMS binding after alkylation of surface receptors with propyl-benzilylcholine mustard (PrBCM). Agonist-induced receptor internalization was determined through the reduction in [^{3}H]NMS binding. Values are means±s.e.mean from three separate experiments, each carried out in quadruplicate.

In unstimulated cells, receptor delivery to the plasma membrane along the synthetic route was linear with a rate constant (k_1) of 22±1 receptors/cell min^{-1}. When cells were pre-treated for 1h with cycloheximide (20 μg ml^{-1}) to block receptor synthesis, receptor recycling to the plasma membrane following carbachol stimulation (0.5 mM for 30 min at 37°C) was found to be exponential, with a rate constant (k_2) of 0.12±0.02 min^{-1}; the recyclable endosomal pool size was 28±1% of the number of receptors initially at the cell surface (11,500/cell). Using the values of k_1 and k_2, the values of k_3 (=0.12±0.01 min^{-1}) and k_4 (=60±6 receptors/cell min^{-1}) were also determined.

Knowing the values for k_1-k_4, we can estimate the extent of receptor trafficking triggered by carbachol stimulation. During a 30-min incubation with carbachol (after pre-treatment with cycloheximide), surface receptor number falls from 11,500 to 4,700/cell. However, the minimum number of receptors that leave the surface during this time is 11,500 x $(1\text{-}e^{-k_3 t})$, or 11,200. Consequently, 4,400 receptors must be recycled. Receptor degradation in 30 min is 1,800/cell; hence, the endosomal pool size at the end of the carbachol incubation will be 5,000. After agonist washout, a further 3,200 receptors will recycle to the plasma membrane. Since $k_2=0.12$ min^{-1}, recycling will be 95% complete after 30 min. During this time a further 1,800 receptors will be degraded, leaving the endosomal pool empty. In short, therefore, receptor traffic is much more extensive than would be predicted from 'snap-shot' measurements of [^{3}H]NMS binding before and after stimulation.

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458P MUSCARINIC M_3 RECEPTOR-MEDIATED PHOSPHOINOSITIDE HYDROLYSIS IN HUMAN CULTURED DETRUSOR SMOOTH MUSCLE CELLS

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In the human detrusor the significant motor innervation is cholinergic; the smooth muscle receptors are muscarinic. Muscarinic receptors have four main subtypes (M_1 - M_4) and it has been shown that M_1 and M_3 receptors are preferentially coupled to phosphoinositide hydrolysis whereas M_2 and M_4 receptor stimulation causes inhibition of adenylate cyclase (Peralta et al. 1988). The aim of this study was to investigate muscarinic receptor-mediated phosphoinositide hydrolysis in human detrusor smooth muscle cells.

Primary cell cultures were established from small biopsies of human detrusor muscle. Accumulation of total [^{3}H]-inositol phosphates was measured on confluent monolayers as described previously (Marsh & Hill 1992). Concentration-response curves to carbachol were obtained and the apparent affinity constants (expressed as pA_2 values) for atropine and four selective muscarinic antagonists were calculated using the method described by Lazareno & Roberts (1987).

Carbachol (10 nM - 1 mM) produced a significant and concentration-dependent rise in the accumulation of total inositol phosphates (EC_{50} = 8.3 +/- 3.1 x 10^{-6} M, mean +/- s.e.m, n = 6 detrusors). The magnitude of the response to 1 mM carbachol was 16.2 +/- 1.2 (n = 6) times that of basal levels. This response was competitively antagonised by atropine (apparent pA_2 value = 9.4 +/- 0.2, n = 5). The potency of selective muscarinic antagonists was measured and gave apparent pA_2 values for 4-diphenylacetoxy-N-methyl-piperidine-methobromide (9.2 +/- 0.3, n = 6), parafluorohexahydrosilafenidol (7.4 +/- 0.2, n = 5), pirenzepine (6.9 +/- 0.4, n = 5) and methoctramine (6.3 +/- 0.1, n = 6). The selective antagonist potency was atropine > 4-diphenylacetoxy-N-methyl-piperidine-methobromide > parafluorohexahydrosilafenidol > pirenzepine > methoctramine and comparison of these pA_2 values with published results (Yang et al. 1991) indicate activation of an M_3 receptor.

These results indicate that human detrusor smooth muscle cells in culture retain their M_3 receptors which are linked to phosphoinositide hydrolysis.

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459P PHARMACOLOGICAL CHARACTERISATION OF MUSCARINIC RECEPTORS MEDIATING CONTRACTION OF HUMAN COLONIC SMOOTH MUSCLE

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Muscarinic receptors have been classified into several subtypes based on the pharmacological evidence of antagonists of known selectivity. In the present study, the muscarinic receptor subtype mediating carbachol-evoked contractions in human colon circular muscle (CM) and longitudinal muscle (LM) of the taenia was investigated.
Strips of macroscopically normal CM and LM from ascending or sigmoid colon were suspended in organ chambers containing Krebs' at 37°C and bubbled with 95% O_2/5% CO_2. Cumulative concentrations of carbachol were added with a contact time of 75 seconds. Following washing, antagonists were incubated for 30 mins before repeating carbachol response curves which in the absence of antagonists were superimposable. Each antagonist was tested at 3-4 concentrations and pA_2 values calculated by Schild plots.
Carbachol (0.01-300μM) contracted CM and LM preparations with EC_{50} values of 11.7 ± 2.3μM (n=8) and 12.6 ± 2.3μM (n=8) respectively.

Table 1 pA_2 values for muscarinic antagonists against carbachol-induced contractions

	Atropine	4-DAMP (M_3)	AF-DX 116 (M_2)	Pirenzepine (M_1)
CM	8.72 ± 0.28	9.41 ± 0.23	7.36 ± 0.43	7.23 ± 0.48
LM	8.60 ± 0.08	9.09 ± 0.16	6.58 ± 0.25	6.87 ± 0.38

(mean ± SEM, n = 4 for each antagonist, slope Schild plots 0.805-1.29)

The slopes of Schild plots for each antagonist were close to unity. In both CM and LM, atropine, a non-selective muscarinic receptor antagonist, and 4-DAMP (M_3) potently inhibited carbachol-evoked contractions whereas AF-DX 116 (M_2) and pirenzepine (M_1) were much less effective (15-100-fold). The rank order of potency of antagonists is 4-DAMP > atropine > AF-DX 116 = pirenzepine in both CM and LM preparations. These studies suggest that in carbachol-stimulated CM and LM, the muscarinic receptors mediating contractions appear to be related to the M_3 subtype.

460P ROLE OF POST-JUNCTIONAL MUSCARINIC M_2 RECEPTORS IN THE CONTROL OF GUINEA-PIG TRACHEAL SMOOTH MUSCLE TONE

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In guinea-pig ileum, after M_3 receptor alkylation, M_2 receptor activation inhibits β-adrenoceptor-mediated relaxation, thereby indirectly contracting histamine pre-contracted tissues (Reddy et al, 1994). In guinea-pig trachea the M_2 antagonist, methoctramine, augments β_2-adrenoceptor-mediated relaxation, in tissues pre-contracted with (+)cis-dioxolane (Watson & Eglen 1994). The aim of the present study was to investigate whether a contractile role for M_2 receptors can be demonstrated in guinea-pig trachea after M_3 receptor alkylation.

Tracheal strips were prepared from male Hartley guinea-pigs (250-350 g) and were suspended under 1 g tension in oxygenated, modified Krebs solution (pH 7.4, 37°C) containing indomethacin (1 μM), tetrodotoxin (1 μM) and corticosterone (30 μM). All values quoted are mean ± s.e. mean (n=5.9). In preparations pre-contracted (3 g) using either oxotremorine M or histamine, the relaxant potency (pEC_{50}) of isoprenaline was significantly different ($p < 0.02$, paired Student's t test) at 7.8 ± 0.1 and 8.4 ± 0.1, respectively. In the presence of methoctramine (0.3 μM) these values were 8.1 ± 0.1 and 8.4 ± 0.1, respectively. These data show that in the absence of M_3 receptor alkylation, activation of M_2 receptors slightly but significantly ($P < 0.02$, paired Student's t test), inhibited the relaxant potency of isoprenaline, in tissues pre-contracted by oxotremorine M.

Second, the effects of M_3 receptor alkylation (1 hr exposure to 40 nM 4-DAMP-mustard) were studied, in the presence of 1 μM methoctramine (to protect M_2 receptors). The potency of oxotremorine M was reduced from 7.4 ± 0.04 to 5.8 ± 0.04, with no significant reduction in the maximum. Post-alkylation contractions were mediated by M_3 receptors, since the affinity (pK_B) of para-fluorohexahydrosiladifenidol (p-F-HHSiD) was 6.7 ± 0.09. In an attempt to reveal indirect contractions due to inhibition of β-adrenoceptor-mediated relaxation (Reddy et al., 1994), after this alkylation procedure, tissues were pre-contracted with histamine (3 μM) and relaxed with isoprenaline (10 nM). Under these conditions the potency of oxotremorine M was 6.0 ± 0.02 and these contractions were not antagonised by methoctramine (1 μM). These data are inconsistent with an interaction at M_2 receptors suggesting that, in contrast to guinea-pig ileum, a contractile role for M_2 receptors could not be revealed.

It is unclear as to the reason for this difference between guinea-pig ileum and trachea. However, these two smooth muscle tissues also differ in their abilities to sustain contractions to muscarinic agonists. One explanation for these data, is that the role of M_2 receptors in modulating β-adrenoceptor-mediated relaxations in trachea is less pronounced than in ileum. Alternatively, an intact population of M_3 receptors may be required to observe M_2 receptor-mediated inhibition of β-adrenoceptor mediated relaxations.

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461P HUMAN EOSINOPHILS AND RECOMBINANT MATRIX METALLOPROTEINASE-2 INCREASE PERMEABILITY OF THE BOVINE AND PORCINE BRONCHIAL MUCOSA

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Addition of stimulated eosinophils from guinea pigs to isolated sheets of bronchial mucosa results in tissue injury and an increased transepithelial flux of tracer that can be inhibited by the antiproteinase α_2-macroglobulin (Herbert *et al.*, 1991), and we have subsequently detected release of the gelatinolytic matrix metalloproteinases (MMP) MMP-2 and MMP-9 during this process. Using [^{14}C] mannitol flux measurements, we now report that human eosinophils reproduce these changes and that they can be mimicked by direct application of MMP-2.

Human peripheral blood eosinophils (purity 92.4 ± 1.9%, n=6) were applied for 16h to the basolateral side of sheets of bovine bronchial mucosa and activated with 5μM A23187. Compared with bronchial mucosa exposed to buffer alone (flux 15.7 ± 1.1 pmol cm^{-2} min^{-1}) or eosinophil-free ionophore solution (flux 16.2 ± 1.3 pmol cm^{-2} min^{-1}), those tissues exposed to 10^7 eosinophils exhibited an increased permeability to mannitol (flux 22.7 ± 2.1 pmol cm^{-2} min^{-1}, n=9, P=0.01). Exposure to 5 x 10^6 cells failed to produce a significant effect (flux 17.9 ± 1.2 pmol cm^{-2} min^{-1}, P=0.2).

Recombinant human pro-MMP-2 was activated by organomercurial (APMA) treatment and dialysed prior to dilution in media containing 0.05% BSA. Neither dialysed APMA nor 0.05% BSA increased the basal flux of mannitol (flux in medium alone 16.1 ± 1.6 pmol cm^{-2} min^{-1}; dialysed APMA 17.5 ± 1.0 pmol cm^{-2} min^{-1}; BSA 15.5 ± 1.2 pmol cm^{-2} min^{-1}). However, incubation for 16h with either 3 or 30u ml^{-1} MMP-2 significantly increased mucosal permeability (fluxes 23.4 ± 2.0 and 24.3 ± 2.3 pmol cm^{-2} min^{-1} respectively; P=0.03, P=0.01; n=8 for each). We also tested MMP-2 in the porcine isovolumic bronchial segment preparation (Omari *et al.*, 1992). MMP-2 (7.2-18.1u ml^{-1}) in the lumen for 2h significantly increased the sensitivity to ACh added intraluminally (EC_{10} pre-exposure 0.34 ± 0.12mM versus post-exposure 0.04 ± 0.02mM, P<0.05, n=6), but not when the ACh was added adventitially. Taken together, these results show that, like eosinophils, MMP-2 increases the permeability of the bronchial mucosa and can thus augment the responsiveness of airway smooth muscle to ACh.

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462P INHIBITION OF HUMAN AIRWAY SMOOTH MUSCLE PROLIFERATION BY DEXAMETHASONE

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An increase in the amount of airway smooth muscle is a characteristic finding in asthmatic airways. Stimulation of proliferation in cultured airway smooth muscle cells has been shown to occur following incubation with foetal calf serum (FCS), histamine and a range of other mediators and growth factors (Hirst & Twort, 1992). Several signal transduction pathways have been implicated in these responses including activation of phospholipase C (PLC). The aims of this study were (i) to investigate the potential for PLC coupled agonists to induce proliferation of cultured human airway smooth muscle, and (ii) to investigate the effect of dexamethasone upon proliferation in these cells.

Human airway smooth muscle cells (passage 4-8) were grown as previously described (Hall et al 1992), and then seeded at about 20% confluence into 96 well plates. Following growth arrest for 24h in medium (DMEM) without FCS, cells were stimulated with a range of agonists in the absence or presence of dexamethasone. After 72-96h, growth was quantified using a proliferation assay (Cell Titer96™, Promega) which utilises cellular conversion of a tetrazolium salt into a coloured formazan compound. Plates were read at 490nm in an ELISA plate reader. Appropriate blank and control wells were included in each individual experiment. Initial experiments were performed using direct cell counting in wells to validate the assay. In each experiment, replicates of 8 wells were used for each condition studied. Results are expressed as mean $\pm$ s.e. compared to control cells in n separate experiments. 10% FCS (dialysed) induced proliferation of human airway smooth muscle cells (response to FCS 164 $\pm$ 8% cf control cells, $n=8$, $p<0.05$). Stimulation of PLC linked receptors known to be present in these cells (Daykin et al 1993) with either 1μM bradykinin ($n=3$) and 0.1mM histamine ($n=5$) failed to induce significant proliferation overall, although in 2 experiments a small response to histamine was observed (121% and 138% cf control cells). Incubation with dexamethasone (1μM) produced a 72 $\pm$ 18% inhibition of FCS-induced proliferation ($n=5$, $p<0.05$).

These results indicate that dexamethasone inhibits the proliferative response of cultured human airway smooth muscle cells to FCS. In addition, the lack of effect upon proliferation of bradykinin, a potent activator of PLC in these cells (Daykin et al 1993), suggests that stimulation of this pathway alone does not induce human airway smooth muscle proliferation.

This work was supported by the National Asthma Campaign.

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463P COMPARISON OF THE EFFECTS OF NS-004 AND SCA40 ON THE ACTIVITY OF SINGLE MAXI-K$^+$ CHANNELS IN BOVINE TRACHEAL SMOOTH MUSCLE CELLS

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The benzimidazolone, NS-004 (3-30 µM), can directly activate maxi-K$^+$ channels in bovine aortic smooth muscle cells and guinea-pig tracheal smooth muscle cells (Olesen and Wätjen, 1992; Olesen *et al.*, 1993). More recently, an imidazo [1, 2a] pyrazine derivative, SCA40 (0.01-10 µM), has been shown to induce relaxation of guinea-pig tracheal smooth muscle (Laurent *et al.*, 1993). This relaxation is inhibited by charybdotoxin, suggesting that the response may involve the activation of maxi-K$^+$ channels. However, it is not known whether SCA40 is able to activate the channel by a direct interaction. In the present experiments, therefore, we have compared the effects of SCA40 and NS-004 on maxi-K$^+$ channel activity in a primary culture of bovine tracheal smooth muscle (BTSM) cells in an attempt to establish whether SCA40, like NS-004, can activate the maxi-K$^+$ channel in this preparation. Conventional techniques were used to record single maxi-K$^+$ channel activity in inside-out, outside-out and cell-attached patches, and the drugs were applied in the bathing medium.

The maxi-K$^+$channel in inside-out patches of BTSM cells had a conductance of 243 ± 8 pS (mean ± s.e. mean; symmetrical 140 mM K$^+$; n = 11) and, in outside-out patches, was blocked by iberiotoxin (100 nM; n = 3). To study the effects of NS-004 and SCA40 on inside-out and outside-out patches the membrane potential was held at +70 mV and the intracellular concentration of Ca^{2+} was set at 10^{-8} M. The application of NS-004 (3-20 µM) to 18 inside-out patches induced a concentration-dependent increase in maxi-K$^+$ channel activity (see Fig. 1). Furthermore, the response to NS-004 did not desensitize: a second application of the compound resulted in a similar level of channel activation. In contrast, SCA40 (0.1 - 10 µM) had no effect on the activity of maxi-K$^+$ channels when applied to either inside-out (n = 13) or outside-out (n = 2) patches of cells which subsequently responded to NS-004 (20 µM). Furthermore, maxi-K$^+$ channel activity recorded in cell-attached mode was not affected by SCA40 (1-10 µM; n = 8).

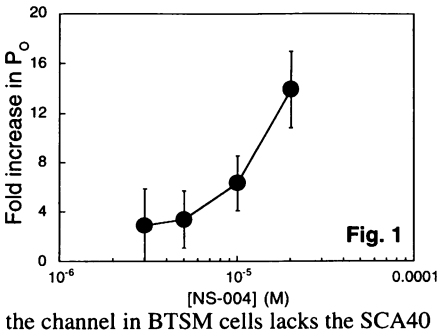


Fig. 1

These results confirm and extend the previous observation that NS-004 induces a direct activation of the maxi-K$^+$ channel. The lack of effect of SCA40 may indicate either that the channel in BTSM cells lacks the SCA40 recognition site or that the compound acts indirectly via a mechanism absent in BTSM cells but present in guinea-pig trachea.

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464P INTRACELLULAR GDP, METABOLIC INHIBITION AND PINACIDIL ACTIVATE GLIBENCLAMIDE-SENSITIVE K-CHANNELS IN SMOOTH MUSCLE CELLS OF RAT MESENTERIC ARTERY

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There are many inconsistencies and much controversy in the literature regarding the ATP-sensitive potassium channel in smooth muscle. Channel conductances ranging from 7~250 pA have been reported, and only a few of the differences can be explained by the different K-gradients used. Many workers have been unable to observe channel activity simply in the absence of ATP. In previous experiments, we found that nucleotide diphosphates (NDPs) could activate a small (24 pS, 60:130 mM K-gradient) conductance channel in rabbit portal vein smooth muscle cells, opened by the potassium channel opener levcromakalim (Beech et al, 1993a,b). In the present experiments, we found that intracellular NDP, metabolic inhibition, and pinacidil can activate the same K-channel in rat mesenteric artery smooth muscle cells; this channel is different from the large conductance K-channel previously reported in rabbit and rat mesenteric artery (Standen et al, 1989). The smooth muscle cells from small (second and third order) rat mesenteric arteries were isolated by using collagenase and pronase. The solutions used for the whole procedure of the dispersion were oxygenated vigorously and continuously and an ice-cold solution was used for tissue dissection to minimize the reduction of intracellular ATP. Conventional whole-cell, cell-attached and isolated patch recording (Hamill et al, 1981) and "perforated patch" recording (Horn & Marty, 1988) were used. After break-through to the whole-cell with an ATP- and NDP-free pipette solution, no detectable current was observed in most of the cells at -37mV. Occasionally (2 out of 8), a small transient outward current developed, which averaged, 6±2pA (mean±s.e.m). Pinacidil (100μM) induced a much larger current, on average at peak, 46±12pA (mean±s.e.m, n=8) and a sustained outward current in all of these cells. When metabolic poisons (cyanide 1mM and 2-deoxy-glucose 10mM) were applied in the bathing solution while in conventional whole-cell recording, no detectable current was observed in four cells. However, metabolic poison induced an outward current, on average of 28±8pA (mean±s.e.m, n=3) during "perforated patch" recording. When 1mM GDP was included in the pipette solution, an outward current slowly developed after breaking through into the whole-cell recording mode and this reached a maximum, on average, of 35±7pA (mean±s.e.m, n=4). The currents induced by pinacidil, metabolic poison and $[GDP]_i$ were all blocked by glibenclamide (10μM).

When inside-out patches were formed into ATP- and NDP-free solution, no characteristic channel activity was observed in 45 patches. However,when GDP (1 mM) or UDP (1 mM) were applied to the inner surface of the membrane, a channel was activated in 9 out of 32 patches. This channel, with the characteristics of 20 pS conductance (60:130 k-gradient), noisy open state, and long open times, is apparently the same channel as that activated by NDP in rabbit portal vein cells. Pinacidil (100μM) increased the channel open probability. In cell-attached patch recording, metabolic poison or pinacidil (100μM) activated a similar channel that had the same channel conductance. Channel activity ceased quickly after inside-out patches were formed in the continuous presence of metabolic poison or pinacidil. The channels activated by NDP, metabolic poison and pinacidil in inside-out and cell-attached patch recordings were reversibly blocked by glibenclamide (10μM).

This work was supported by the British Heart Foundation.

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465P EFFECT OF THE POTASSIUM CHANNEL OPENERS CROMAKALIM, PINACIDIL AND RP49356 ON THE GUINEA-PIG ISOLATED DIAPHRAGM

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Cromakalim, pinacidil and RP49356 have previously been shown to open ATP-sensitive potassium channels (K_{ATP}) in excised patches from mouse skeletal muscle (Weik and Neumcke, 1990). The purpose of the present study was to investigate the functional consequences of K_{ATP} opening in skeletal muscle.

Strips of guinea-pig diaphragm were attached to electrodes for direct electrical stimulation. Tension was recorded isometrically. Resting tension was set initially to 5g. The effects of potassium channel openers were assessed against non-fatiguing function (twitch contractions; 0.1Hz, 0.5ms duration, supramaximal voltage), fatiguing function (intermittant tetanic contractions; trains of pulses at 30Hz, 1TPS, 300ms following metabolic inhibition with 2-deoxy-glucose/N_2) and against the development of contracture (continued high frequency stimulation, 30Hz for 330ms once per sec in metabolically inhibited preparations).

Cromakalim (10-100μM) had no significant effect on twitch contractions or initial tetanic tension following metabolic inhibition. On continued intermittant tetanic stimulation in the presence of vehicle, tension declined (fatigue) with half maximal fall in tension ($t_{1/2}$) being reached after 1.51 ± 0.12 min (n=15). $t_{1/2}$ was significantly shortened in the presence of cromakalim. $t_{1/2}$ values were 77.3 ± 11.8% (n=5; p>0.05), 65.9 ± 5.3% (n=4; p< 0.01) and 53.7 ± 7.9% (n=6; p<0.01) of vehicle control $t_{1/2}$ in the presence of 10, 30 and 100μM cromakalim, respectively. Guinea-pig diaphragm preparations stimulated to exhaustion in the presence of vehicle developed a contracture (equivalent to 27.5% twitch tension) in 25.8 ± 1.4 min (n=15). Time to contracture was delayed by cromakalim. Times to contracture were 27.6 ± 3.0 min (n=4), 38.8 ± 0.8 min (n=4; p<0.01) and 61.2 ± 3.6 min (n=6; p<0.01) in the presence of 10μM, 30μM and 100μM cromakalim, respectively.
In similar experiments neither pinacidil (10-100μM) nor RP49356 (10-100μM) significantly inhibited twitch contractions or initial tetanic tension following metabolic inhibition in the guinea-pig diaphragm. Both agents, however, shortened $t_{1/2}$. $t_{1/2}$ values were 83.5 ± 7.0% (n=5; p>0.05), 92.9 ± 11.0% (n=3; p>0.05) and 67.0 ± 2.9% (n=4; p<0.05) of vehicle control $t_{1/2}$ in the presence of 10μM , 30μM and 100μM pinacidil, respectively and 114 ± 2.6% (n=5; p>0.05), 94.0 ± 4.5% (n=6; p>0.05) and 75.7 ± 1.8% (n=5; p<0.05) of vehicle control $t_{1/2}$ in the presence of 10μM , 30μM and 100μM RP49356, respectively. Pinacidil and RP49356 also delayed contracture in the metabolically exhausted guinea-pig diaphragm. Times to contracture were 18.6 ± 1.0 min (n=16), 26.0 ± 2.2 min (n=5, p<0.05), 32.1 ± 2.6 min (n=4, p<0.05) and 32.7 ± 2.6 min (n=4, p<0.01) in the presence of vehicle, 10μM, 30μM and 100μM pinacidil, respectively and 17.2 ± 0.5 min (n=12), 22.0 ± 1.9 min (n=5, p<0.05), 27.0 ± 1.4min (n=6, p<0.01) and 32.8 ± 0.8min (n=5, p<0.01) in the presence of vehicle, 10μM, 30μM and 100μM RP49356, respectively.

In conclusion, opening K_{ATP} appears to mediate a selective inhibition of mechanical function in the metabolically compromised isolated guinea-pig diaphragm preparation. K^+-channel opener-induced depression of function in metabolically compromised skeletal muscle cells is associated with a delay in the appearance of the signs of metabolic exhaustion.

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466P EFFECTS OF CETIEDIL AND ITS ANALOGUES ON THE Ca^{2+}-ACTIVATED K^+ PERMEABILITY OF RABBIT ERYTHROCYTES AND ON LEVCROMAKALIM-STIMULATED $^{86}Rb^+$ EFFLUX FROM RAT AORTA

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Cetiedil (the 2-(N-hexahydroazepinyl)ethyl ester of α-cyclohexyl-α-(3-thienyl) acetic acid) was first developed as a peripherally acting vasodilator and has the additional interest that it blocks several K^+ channels including the Ca^{2+} activated K^+ channel ($K_{(Ca)}$) of erythrocytes (Christophersen & Vestergaard-Bogind, 1985), and the cell volume-sensitive K^+ channel of lymphocytes (Sarkardi *et al.*, 1985) and hepatocytes (Sandford *et al.*, 1992). In the present study we have assessed the ability of cetiedil and some novel compounds related to it to block the rabbit erythrocyte $K_{(Ca)}$ and the K^+ permeability acted on by levcromakalim in rat aorta.

The effects of test compounds on $K_{(Ca)}$ were studied using extracellular K^+-sensitive electrodes to measure the net loss of K^+ which results from the application of the Ca^{2+} ionophore A23187 to rabbit erythrocytes suspended in a physiological solution containing less K^+ than normal (0.1 - 0.15 mM). The same compounds were tested for their effects on the increase in $^{86}Rb^+$ efflux that results from the application of levcromakalim (10 μM) to rat aorta; here a normal concentration (4.6 mM) of K^+ was used.

Cetiedil inhibited A23187-stimulated K^+ loss from rabbit erythrocytes with an IC_{50} of 26 ± 3 μM (fitted value ± s.d. from least squares curve fit: data from 36 observations in 6 expts). Synthesis of congeners of cetiedil showed that activity was strongly correlated with lipophilicity. Thus UCL 1285 (cetiedil methiodide, a quaternary derivative) caused no significant inhibition at 100 μM, whereas the most potent compound was the highly lipophilic UCL 1495 (the 2-[N-(5-ethyl-2-methylpiperidino)]ethyl ester of α,α,α-triphenylacetic acid) which had an IC_{50} of 1.2 ± 0.1 μM (6 obs. in 3 expts.)

Cetiedil, UCL 1285 and UCL 1495 were also tested on the rat aorta. In contrast to the results obtained with erythrocytes, cetiedil inhibited levcromakalim-stimulated $^{86}Rb^+$ efflux with an IC_{50} of 1.4 ± 0.4 μM (29 obs. in 7 expts.), UCL 1285 with an IC_{50} of 3.7 ± 2.4 μM (18 obs. in 6 expts) whereas UCL 1495 had no significant effect at 10 μM (3 obs. in 3 expts.).

The different relative potencies observed with these agents show that the structural requirements for block of the two K^+ permeabilities are quite distinct.

Supported by the Wellcome Trust. We thank Smith Kline Beecham for the kind gift of levcromakalim.

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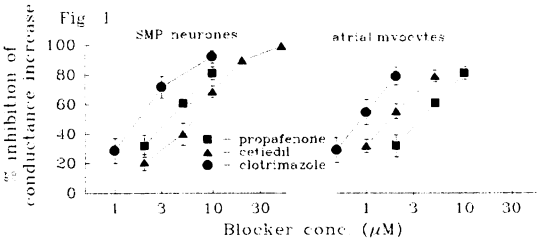
467P INHIBITORS OF G-PROTEIN-ACTIVATED K^+ CONDUCTANCES IN RAT ATRIAL MYOCYTES AND GUINEA-PIG SUBMUCOUS PLEXUS NEURONES

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Potassium channels are often characterized by their sensitivity to blocking agents. In this study we compare the relative potencies of three drugs to inhibit agonist-induced increases in K^+ conductance in rat atrial myocytes and neurones from the submucous plexus (SMP) of guinea-pig caecum. The compounds tested were cetiedil, an antisickling agent, which has been shown to block the calcium-activated potassium channel (K_{Ca}) in erythrocytes (Christophersen & Vestergaard-Bogind. 1985) and volume-activated K^+ channels in rat hepatocytes (Sandford *et al.*,1992), propafenone. which has been reported to inhibit K_{ACh} in guinea-pig atrial myocytes (Inomata *et al.*,1993) and clotrimazole. an antifungal agent recently shown to inhibit K_{Ca} in erythrocytes (Alvarez *et al.*,1992).

Cells were isolated with the aid of collagenase and trypsin from tissues of adult guinea-pigs and 1-day old rats and kept for up to 3 days in tissue culture. Cells adhering to the base of petri dishes were superfused with a Hepes buffered physiological saline at 30°C. Membrane potential was recorded with an intracellular microelectrode and input resistances estimated by the injection of current pulses via a 'bridge' circuit.

Increases in K^+ conductance were induced in SMP neurones by 1μM noradrenaline and in atrial myocytes by 1 μM adenosine. Both types of cell also showed a substantial hyperpolarization which exhibited only modest desensitization during the 1 - 1.5 min application. The effect of blockers on agonist responses were determined after a 5 min exposure. each concentration being tested on at least 4 cells. Each of the drugs inhibited the K^+ conductance increase in both cell types (see Fig. 1). The possibility that the inhibition observed reflected receptor rather than channel block was ruled out for cetiedil in SMP neurones by showing that it was equally effective in inhibiting the sustained conductance increase induced by intracellular dialysis with GTP-γ-S.



In the presence of clotrimazole. and to a lesser extent propafenone. the agonist-induced conductance increase showed a more pronounced 'fade', suggestive of open channel block. A Hill equation was fitted to the inhibition curves. using a non-linear least-squares procedure. and provided the estimates of IC_{50} shown in table 1. Clotrimazole is the most potent blocker of the three in both preparations. Cetiedil is more effective than propafenone in myocytes but less so in SMP neurones.

The differing order of potency for these blocking agents suggests that the K^+ channels activated by G-protein in these two cell types may be different.

Table 1	IC_{50} (μM) fitted values ± s.d.	
	SMP neurones	atrial myocytes
clotrimazole	1.74 ± 0.13	0.88 ± 0.01
cetiedil	5.87 ± 0.37	1.75 ± 0.06
propafenone	3.53 ± 0.26	3.33 ± 0.56

These studies were supported by the Wellcome Trust.

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468P THE EFFECT OF Rb^+-SUBSTITUTED KREBS SOLUTION ON THE ELECTRICAL AND MECHANICAL ACTIVITY OF GUINEA-PIG ISOLATED TRACHEALIS MUSCLE AND ON TRACHEAL RESPONSES TO RELAXANT AGENTS

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Krebs solution in which K^+ is substituted by Rb^+ (RbPSS) antagonises the relaxant effects of cromakalim in both guinea-pig trachealis muscle and in rat aorta. Such antagonism has been attributed to Rb^+-induced blockade of the K^+-channel (K_{KCO}) opened by cromakalim (Foster *et al.*, 1992; Greenwood & Weston, 1993). We have now further explored the selectivity and mechanism of action of RbPSS in antagonising bronchodilator drugs acting on guinea-pig trachealis muscle.

Tissue bath recording of tension changes showed that isoprenaline (1nM-0.3μM), levcromakalim (0.1-10μM) and theophylline (1μM-1mM) each caused concentration-dependent suppression of the spontaneous tone of the trachea. RbPSS increased the spontaneous tone of the trachea (25.9 ± 5.0%; mean ± s.e.mean), profoundly depressed the log concentration-effect curve for levcromakalim and caused minor antagonism of isoprenaline and theophylline (approximately threefold in each case). Nifedipine (1μM) did not modify the actions of isoprenaline or theophylline as observed in normal Krebs solution and did not potentiate levcromakalim. However, this agent prevented the antagonism of isoprenaline and theophylline provided by RbPSS. In contrast, nifedipine did not prevent RbPSS-induced antagonism of levcromakalim.

Simultaneous intracellular recording of membrane potential and mechanical activity (Dixon & Small, 1983) showed that RbPSS increased the mechanical tone of the tissue and converted spontaneous electrical slow waves into regenerative action potentials. The ability of levcromakalim (10μM) to relax the trachealis muscle and to cause cellular hyperpolarisation were both markedly attenuated by RbPSS (1.34 ± 0.3g and 27.5 ± 2.3mV in Krebs solution; 0.35 ± 0.1g and 10.0 ± 2.7mV in Rb PSS). RbPSS had little effect against the relaxant activity of isoprenaline (1μM) but markedly reduced the cellular hyperpolarisation induced by the agonist at β-adrenoceptors (1.69 ± 0.38g and 16.0 ± 1.2 mV in Krebs solution; 1.86 ± 0.29g and -0.86 ± 1.5 mV in RbPSS).

It is concluded that Rb^+ substitution of K^+ in Krebs solution promotes an increase in tracheal tone which is associated with the discharge of regenerative action potentials and hence an increase in the cellular influx of Ca^{2+}. Inhibition of the relaxant actions of isoprenaline and theophylline observed in RbPSS reflects functional antagonism attributable to promotion of Ca^{2+} influx through nifedipine-sensitive channels. In contrast, the equivalent inhibition of the relaxant action of levcromakalim may reflect blockade by Rb^+ of K_{KCO}. Voltage-dependent inhibition of Ca^{2+} influx through nifedipine-sensitive, L-type Ca^{2+}-channels is unlikely to play a major role in mediating the tracheal relaxant action of levcromakalim. The present findings also support the suggestion of Small *et al.* (1993) that cellular hyperpolarisation is not crucial for the tracheal relaxant action of isoprenaline.

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469P EVIDENCE THAT K^+-CHANNEL OPENING IS NOT IMPORTANT IN THE TRACHEAL RELAXANT ACTION OF SCA 40

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Largely on the basis of antagonism by charybdotoxin and by a K^+-rich medium, Laurent *et al.* (1993) have suggested that the opening of large, Ca^{2+}-dependent K^+-channels (BK_{Ca}) is important for the action of SCA 40 (6-bromo-8-methylaminoimidazo[1,2-a] pyrazine - 2-carbonitrile) in relaxing guinea-pig trachealis muscle. We have put this hypothesis to the test by tissue bath studies of guinea-pig trachealis together with simultaneous measurements of membrane potential and mechanical activity as described by Dixon and Small (1983).

SCA 40 (1nM-10μM), isoprenaline (1nM-300nM) and levcromakalim (100nM-10μM) each produced concentration-dependent suppression of the spontaneous tone of the trachea. A K^+-rich (80mM), but isosmolar, Krebs solution antagonised SCA 40 (approximately 60 fold), isoprenaline (approximately 20 fold) and ablated the log concentration-effect curve for levcromakalim. Charybdotoxin (100nM) antagonised SCA40 (approximately 4 fold) and isoprenaline (approximately 3 fold). Nifedipine (1μM) did not itself modify the relaxant actions of SCA 40, isoprenaline or levcromakalim. However, nifedipine (1μM) prevented the antagonism of SCA 40 and isoprenaline induced by the K^+-rich medium but did not prevent the equivalent antagonism of levcromakalim. Nifedipine (1μM) also prevented the antagonism of SCA 40 and isoprenaline provided by charybdotoxin (100nM). Electrophysiological experiments revealed that SCA 40 (1μM) was able to cause full suppression of the spontaneous tone of guinea-pig trachealis muscle without significant change in membrane potential. At a concentration of 10μM (supramaximally-effective in suppressing spontaneous mechanical tone) SCA 40 suppressed electrical slow waves and evoked 19.0 ± 2.7mV (mean ± s.e.mean; n = 4) hyperpolarisation.

The present findings support the observation of Huang *et al.* (1993) that the inhibition of salbutamol-induced relaxation provided by charybdotoxin, or by a K^+-rich medium, represents functional antagonism attributable to promotion of Ca^{2+} influx through L-type channels. The antagonism of SCA 40 by charybdotoxin and a K^+-rich medium has a similar basis. The ability of nifedipine to prevent antagonism of SCA 40 by either charybdotoxin or the K^+-rich medium, together with the failure of SCA 40 (1μM) to cause cellular hyperpolarisation suggests that the opening of BK_{Ca} channels with consequential voltage-dependent inhibition of Ca^{2+} influx through L-type channels is not important for the relaxant activity of SCA 40. The ability of the K^+-rich medium to ablate the relaxant activity of levcromakalim and the failure of nifedipine to modify the action of levcromakalim suggest that any voltage-dependent inhibition of Ca^{2+} influx caused by this agent involves channels distinct from the nifedipine-sensitive, L-type channel.

S.J.C. was supported by Sandoz Pharma Ltd.

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470P ANALYSIS OF THE PROPRANOLOL-SENSITIVE RELAXATION TO ISOPRENALINE IN RAT DISTAL COLON

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Isoprenaline mediated relaxation of rat distal colon longitudinal smooth muscle is mediated largely via atypical (β_3-) adrenoceptors (McLaughlin & MacDonald, 1990). A small component of the response to isoprenaline is sensitive to propranolol and it is difficult to determine whether this effect of propranolol is due to an effect on β_1/β_2 or β_3-adrenoceptors although a previous study suggested that β_1-adrenoceptors were not invoved (MacDonald & Lamont, 1993). We report further experiments using wider concentration ranges of antagonists in an attempt to answer the question.

Longitudinal segments of rat colon were suspended in Krebs solution at 37°C for isometric recording. The Krebs solution contained EDTA (30 μM), ascorbic acid (30 μM) and prazosin (0.1 μM) and was gassed with 95/5% O_2/CO_2. After an initial equilibration period, reproducible contractions to a submaximal concentration of methacholine (0.3 μM) were obtained before carrying out a concentration response curve (CRC) to isoprenaline in a non-cumulative manner. Up to four consecutive CRCs to isoprenaline were carried out in each tissue with a 1h interval between each curve. Antagonists were present in increasing concentrations during the intervals between CRCs. Control tissues received no antagonist to allow estimation of the magnitude of time-dependent changes. A total of six different concentrations of propranolol and the selective β_1- and β_2-adrenoceptor antagonists CGP 20712A and ICI 118,551 respectively were examined over the range 0.003 - 1 μM. In separate experiments the effects of a combination of ICI 118,551 (0.3 μM) and CGP 20712A (0.1 μM) were compared with the effects of the individual antagonists.

Propranolol produced a small (5 - 6 fold) significant shift of the isoprenaline CRC at concentrations 0.003 - 0.03 μM. Larger shifts were produced by 0.3 μM (14 fold) and 1 μm (18 fold). CGP 20712A produced a small (3 - 4 fold) significant shift at concentrations 0.01 - 1 μM. ICI 118,551 also produced small non-significant shifts (2 - 3 fold) at concentrations of 0.1 to 1 μM. A combination of ICI 118,551 (0.3 μM) and CGP 20712A (0.1 μM) produced a 13 fold shift, a significantly greater shift than expected from the individual shifts. The shift produced by the combination of antagonists was slightly less than that produced by 1 μM propranolol (18 fold).

In conclusion, small β_1- and β_2-adrenoceptor mediated components of the response to isoprenaline in rat distal colon were detected. A combination of β_1- and β_2-blockade produced a greater effect than expected from the effects of individual blockade. The effects of propranolol appear to be due to a combination of β_1- and β_2-adrenoceptor blockade although an additional action of propranolol at β_3-adrenoceptors cannot be ruled out.

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471P ATYPICAL β-ADRENOCEPTORS IN A VASCULAR SMOOTH MUSCLE PREPARATION

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Atypical ß-adrenoceptors ($ß_3$) mediating smooth muscle relaxation (Bond & Clarke 1988; Bianchetti & Manara, 1990; McLaughlin & MacDonald 1990; de Boer et al 1993) and lipolysis (Arch et al 1984) have been described. It is however not known whether such atypical ß-adrenoceptors exist in vascular smooth muscles. In this study, the effect of propranolol on vascular smooth muscle relaxation produced by selective (CGP 12177, Salbutamol and BRL 37344) and non-selective (Isoprenaline) ß-adrenoceptor agonists was examined.

Ring segments (4mm) of the common carotid artery from Sprague-Dawley rats (150-200g) of either sex were mounted in Krebs' solution (37°C, 95% O_2 / 5% CO_2) under 1g tension. To obtain relaxant responses, the tissues were precontracted with noradrenaline(0.1μM). Thereafter, agonist concentration-response curves were obtained by cumulative dosing in the absence or presence of propranolol (30 min. incubation).

Isoprenaline produced a concentration-dependent relaxation with an EC_{50} value of 89 ± 17nM. Propranolol displaced the isoprenaline curve to the right without reducing the E_{max}. The Schild plot yielded a straight line with a slope (0.74 ± 0.10) significantly less than 1 indicating interaction with sites other than $ß_1$- and $ß_2$-adrenoceptors. CGP 12177, Salbutamol and BRL 37344 also relaxed ring segments of the carotid artery. EC_{50} values were 3.4 ± 0.5 μM, 9.2 ± 1.6 μM and 58 ± 6.0 μM respectively. The relaxations produced by these agonists were however not antagonized by propranolol (0.1 μM). This concentration of propranolol reduced the potency of isoprenaline approximately 30-fold.

These observations would suggest that atypical ß-adrenoceptors ($ß_3$?) also exist in this vascular smooth muscle preparation.

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472P EVIDENCE THAT BUSPIRONE POTENTIATES THE VAGAL BRADYCARDIA INDUCED BY UPPER AIRWAY STIMULATION IN ANAESTHETISED RABBITS

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5-HT_{1A} receptors modulate the excitability of cardiac vagal motoneurones (CVM's) and consistent with this, in anaesthetized rabbits, the activation of CVM's by the "diving response" is potentiated by buspirone (Futuro-Neto *et al.*, 1993). The "diving response" is due to the interaction of upper airway receptors and chemoreceptors (Daly & Angell-James,1979) and also causes a rise in blood pressure and an apnoea. Experiments were carried out to determine whether activation of CVM's (by analyzing R-R interval at 3s) and the apnoea evoked by stimulation of upper airway receptors alone are potentiated by buspirone and to determine (by analyzing peak R-R interval) if peripheral chemoreceptors contribute to this by repeating these experiments in hyperoxic animals so that Po_2 is maintained above normal during the apnoea.

Rabbits were anaesthetized with urethane i.v. 1.5g kg^{-1}. Simultaneous recordings were made of blood pressure, renal (RNA) and phrenic nerve activity (PNA; Shepheard et al., 1990). ECG was also recorded from which changes in R-R interval were calculated and arterial blood gases were monitored. Bi-directional tracheal cannulation was performed to allow either spontaneous ventilation with air or oxygen enriched air and the independent delivery of herbal cigarette smoke to the upper airways. Smoke volume (10-50 ml) was chosen to produce a sub maximal bradycardia. Atenolol (1mg kg^{-1}) was then administered i.v. and the smoke challenge repeated every 10 min until a stable bradycardia was observed. 5 min after the stable smoke challenge saline or buspirone (200μg kg^{-1}) was administered i.c. (20 μl over 20s) and the smoke challenges repeated for another 25 min. Time matched comparisons of buspirone induced changes in the reflex with that of vehicle were made by ANOVA.

15 min after the administration of buspirone (n=4) there was a significant ($P < 0.05$) potentiation of the changes in both 3s and peak R-R interval by 21 ± 5 ms and 61± 23 ms (mean ± s.e.mean) compared with that of saline (n=4) 1 ± 2 and -6 ± 10 ms. The duration of the apnoea was unaffected, 18 ± 7 s compared to 16 ± 3 s after saline. In addition, buspirone failed to affect the increase in RNA (412 ± 107% c.f. 306 ± 55%) or BP (23 ± 4 c.f. 19 ± 3 mmHg). In hyperoxic animals (Po_2 180-200 mmHg which never fell below 120 mmHg by the end of the apnoea) buspirone (n=4) again significantly potentiated the changes in 3s and peak R-R interval by 38 ± 11 ms and 103 ± 4 ms compared with that of saline (n=4). The duration of the apnoea was again unaffected 18 ± 7s compared to 17 ± 4s for saline. These results indicate that 5-HT_{1A} receptors are involved in the reflex activation of CVM's by stimulation of the upper-airways and that this does not involve peripheral chemoreceptors.

S.B.D. is in receipt of a British Heart Foundation PhD Studentship. We wish to thank Mr S.Wilkinson for technical assistance

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473P FURTHER EVALUATION OF PUTATIVE 5-HT_{2A} RECEPTORS MEDIATING RELAXATION OF GUINEA-PIG TRACHEA

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On the basis of sensitivity to ketanserin, it has been proposed that 5-HT_{2A} receptors mediate both contraction and relaxation of guinea-pig trachea (Baumgartner *et al.*, 1992). In the present study we have further evaluated the nature of the putative relaxant 5-HT_{2A} receptor in this preparation.

Proximal tracheal segments were obtained from male Dunkin Hartley guinea-pigs (400-500g) and cut open to form flaps of tissue 3 mm wide from which the epithelium was mechanically removed. Tissues were suspended for isometric recording under an initial tension of 1.0 g in oxygenated (95% O_2 / 5% CO_2) Krebs solution at 37°C. All experiments were conducted in the presence of indomethacin (3.0 μM) and atropine (1.0 μM) to preclude involvement of prostanoids and a cholinergic component of the response to 5-HT. In most experiments tissues were also exposed to pargyline (100 μM for 15 minutes followed by washout). Antagonists were evaluated after a one hour equilibration period.

Cumulative addition of 5-HT (0.01 - 30 μM) evoked concentration-dependent, bell shaped concentration-effect curves similar to those observed in other investigations (Baumgartner *et al.*, 1992). In tissues that had been pre-contracted with an approximate pEC_{50} concentration of histamine (3.0 μM), a bell shaped curve was still evident with 5-HT (0.03 - 3.0 μM) inducing a further contraction and relaxations at higher concentrations (≥3.0 μM). Ketanserin (0.1 μM), but not spiperone (1.0 μM) caused relaxation in its own right. Spiperone (1.0 μM, n=4) abolished the contractile phase isolating the relaxant response to 5-HT (pEC_{50} [95% C.L.] = 5.1 [4.8-5.3], n=8) and was included in all subsequent experiments. In tissues pre-contracted with the thromboxane mimetic U46619 (0.03 μM), ketanserin did not cause relaxation and did not attenuate relaxant responses to 5-HT. The magnitude of the relaxant responses to 5-HT were variable ranging from 40-100%. Many tissues failed to maintain tension on exposure to histamine. Tissues which were not exposed to pargyline were capable of maintaining histamine evoked contractions but the relaxant response to 5-HT was abolished. Relaxant responses to 5-HT were not significantly different after pre-treatment of animals with para-chlorophenylalanine (150 mg kg^{-1} ip for 3 days, n=4) but were absent in tissues taken from reserpine treated animals (10 mg kg^{-1} ip, 18 h prior to use, n=4) and in tissues exposed to guanethidine (1.0 μM). In tissues exposed to either propanolol (1.0 μM) or atenolol (1.0 μM) but not prazosin (1.0 μM), relaxations induced by 5-HT were abolished. Relaxations to 5-HT were mimicked by tyramine (pEC_{50} [95% C.L.] = 5.8 [5.6-6.0], n=4) and responses to tyramine were abolished by propranolol (1.0 μM).

These data suggest that the relaxant response to 5-HT in guinea-pig trachea is mediated indirectly via the release of endogenous noradrenaline and subsequent activation of β-adrenoceptors. The reason for the discrepancies between these studies and those of Baumgartner *et al.*, (1992) is not known. However, the failure of both ketanserin and spiperone to antagonise the relaxant response to 5-HT suggests that 5-HT_{2A} receptors are not involved in the 5-HT mediated relaxant response in this preparation of guinea pig trachea.

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474P DIFFERENCES IN THE EFFECTS OF KETANSERIN ON 5-HT-RECEPTOR-MEDIATED RESPONSES IN RABBIT SAPHENOUS VEIN AND GUINEA-PIG JUGULAR VEIN

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Ketanserin (a supposedly 5-HT_{2A}-receptor selective antagonist) has been shown to inhibit contractile responses evoked by sumatriptan (Martin *et al.*, 1990), an agonist which is devoid of 5-HT_2-receptor activity (Humphrey *et al.*, 1988), in rabbit saphenous vein (RSV) a tissue containing 5-HT_{1D}-receptors. In this study, we compared the effects of ketanserin on 5-HT and/or sumatriptan in RSV and guinea-pig jugular vein (GPJV).

Isolated tissues from New Zealand White rabbits (2-2.5kg) and Dunkin Hartley guinea-pigs (250-300g) were mounted for isometric tension recordings in organ baths perfused with Krebs physiological salt solution, aerated with 95% O_2/5% CO_2 and maintained at 37°C. In RSV, two cumulative concentration-effect curves (CEC) to 5-HT or sumatriptan were performed in the same tissue either in the absence or presence of ketanserin (1,3 or 10μM) or nifedipine (0.3 and 3nM; 30min equilibration period). In GPJV (pre-contracted with the thromboxane mimetic, U-46619, 30nM), sumatriptan produced an endothelial-dependent relaxation. To avoid 5-HT-receptor desensitisation matched pairs from the same animal were used. Effects of ketanserin and nifedipine on voltage-operated calcium channels in guinea-pig ileum (GPI) were assessed as described by Rupniak *et al.* (1993).

The pEC_{50} values for 5-HT and sumatriptan in RSV (responses calculated as percentage maximum contraction) were 7.6±0.1 (n=18) and 6.2±0.04 (n=21), respectively. In RSV, the concentration ratio (CR) for sumatriptan and ketanserin (1μM) was 6.52±1.5; full Schild plots for ketanserin were obtained and the pA_2 was 6.75 (C.L. 6.68-6.82) and 6.76 (C.L. 6.58-6.93) using 5-HT (slope=0.8, C.L. 0.6-1.0) and sumatriptan (slope=0.8, C.L. 0.5-1.1), respectively. In GPJV (responses calculated as percentage Ach-induced relaxation), sumatriptan had a pEC_{50} value of 6.3±0.2 (n=16); the pK_B value for methiothepin (10nM) using this agonist was 9.0±0.3 (n=4), whereas, ketanserin (1μM) had no effect (CR >2, n=4). In GPI, both ketanserin and nifedipine inhibited Ca^{2+}-entry to give pK_B values of 6.5±0.3 (n=4) and 10.5±0.7 (n=6), respectively. Nifedipine (3nM) had no effect on sumatriptan-induced CECs in RSV (n=4).

Although ketanserin has weak Ca^{2+}-channel blocking properties, its effects on sumatriptan-induced contractions in RSV probably reflects antagonism of 5-HT_{1D}-receptors. It is possible that the differential effects of ketanserin on 5-HT_{1D}-receptor mediated responses in GPJV and RSV may reflect the existence of 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$-receptor subtypes.

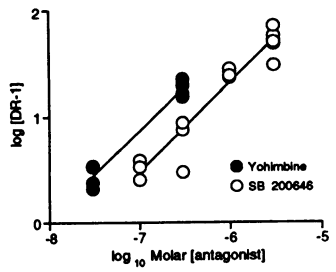
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475P 5-HT_{2B} RECEPTORS MEDIATE CONTRACTION OF RAT STOMACH FUNDUS CIRCULAR MUSCLE

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It has been proposed that 5-HT_{2B} receptors mediate the contractile response of rat stomach fundus longitudinal muscle (*see* Humphrey *et al.*, 1993). In the present study we investigate the nature of an as yet un-classified 5-HT receptor which is present in the circular muscle (RSF-CM) of the rat stomach fundus (Vane 1957).

Whole stomachs were obtained from male Sprague Dawley rats (250-350g) and strips of tissue (1-1.5 x 15 mm) were cut perpendicular to the greater curvature of the fundus to functionally isolate the circular muscle. Muscle strips were mounted for isotonic recording under a load of 1.5 g in oxygenated (95% O_2 / 5% CO_2) Tyrodes solution at 37°C. All experiments were conducted in the presence of indomethacin (3.0 μM). Preliminary experiments indicated that removal of the mucosa and exposure of tissues to pargyline (100 μM for 15 minutes followed by washout) was necessary to both reduce variability, and increase tissue sensitivity to agonists. Two cumulative concentration-effect curves were constructed to 5-HT in each preparation, the first in the absence of antagonist, and the second after incubation with the antagonist for at least one hour.



Cumulative addition of 5-HT evoked reproducible, concentration-dependent, contractile responses in RSF-CM and yielded monophasic concentration-effect curves (pEC_{50} [95% C.L.] = 8.14 [7.81-8.54], n=27). The 5-HT_{2B}/5-HT_{2C} selective antagonist SB 200646 (0.1 - 3 μM), and yohimbine (0.01 & 0.3 μM) which discriminates 5-HT_{2B} from 5-HT_{2C} receptors (*see* Baxter *et al* ., 1994), acted as surmountable antagonists of 5-HT in RFS-CM (pA_2 estimates [slope] of 7.6 [0.84] and 8.1 [0.82], n = ≥ 3, respectively, *Figure 1*). Rauwolscine (0.1 & 0.3 μM) acted as a potent but non-surmountable antagonist of 5-HT in RFS-CM yielding concentration-ratios ± s.e.m. of 17.9 ± 1.3 and 39.6 ± 8, n=4 and intrinsic activities ± s.e.m. (relative to control maximum response) of 0.84 ± 0.1 and 0.78 ± 0.1, n=4 respectively. Neither ketanserin (1.0 μM, n=4) nor spiperone (1.0 μM, n=4) caused antagonism of responses to 5-HT in RFS-CM.

These data, obtained using antagonist probes, are consistent with the presence of 5-HT_{2B} receptors mediating contractile responses to 5-HT in RSF-CM.

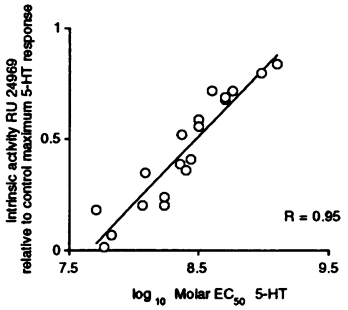
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476P VARIABILITY IN 'EFFECTIVE RECEPTOR RESERVE' AT PUTATIVE 5-HT $_{2B}$ RECEPTORS IN RAT STOMACH FUNDUS

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It has been proposed that 5-HT$_{2B}$ receptors mediate contraction in longitudinal muscle of rat stomach fundus (*see* Humphrey *et al.*, 1993). In the present study the influence of variations in effective receptor reserve on the concentration-effect relationships for 5-HT and low efficacy (partial) agonists has been investigated in this preparation.

Tissues were set up as described previously (Baxter *et al.*, 1994). Two agonist concentration-effect curves were constructed in each preparation. In one protocol, the same agonist was used before and after inactivation of putative 5-HT$_{2B}$ receptors using phenoxybenzamine (3.0 μM for 15 minutes followed by washout). In the second protocol a first curve was constructed to 5-HT and a second to the low efficacy agonist, RU 24969 (*see* Baxter *et al.*, 1994). First and second curves were then fitted, simultaneously, to the Operational Model of Agonism (Black & Leff 1993).



Exposure of tissues to phenoxybenzamine resulted in a variable reduction, of 30-80%, in the maximum response to 5-HT. The reduction in maximum response induced by phenoxybenzamine could be attenuated by co-incubation with the 5-HT$_{2B}$ antagonist, yohimbine (1 μM). Operational analysis yielded mean τ and mean $pK_A \pm$ s.e.m. estimates of 10.9 ± 3.7 and 8.0 ± 0.2, n=8, respectively and indicates that 5-HT does not reach the theoretically achievable maximum response in this system. The large error on τ reflects the variability observed in individual estimates (range 3.2 - 28.6). RU 24969 was a partial agonist with respect to 5-HT (pEC_{50} [95% C.L.] = 7.02 [6.81-7.25], intrinsic activity ± s.e.m. = 0.42 ± 0.10, n=19). The intrinsic activity for RU 24969 varied from one preparation to another (range 0-0.85) but correlated well with pEC_{50} estimates for 5-HT determined in the same preparation (Figure 1, R=0.95. $p \leq 0.05$). Operational analysis (using K_A for 5-HT determined above), yielded a mean $pK_A \pm$ s.e.m. of 6.9 ± 0.1 and mean $\tau \pm$ s.e.m of 0.99 ± 0.2 for RU 24969 and a $\tau \pm$ s.e.m. estimate for 5-HT of 3.62 ± 0.81, n = 17.

These data suggest inter-tissue differences in sensitivity to receptor inactivation and to low efficacy agonists may be explained by variations in effective receptor reserve. Neither the cause, nor the nature (ie. resulting from variation in receptor number or in efficiency of transduction) of this variability is known.

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477P 5-HT$_{2B}$-LIKE RECEPTORS MEDIATE ENDOTHELIUM DEPENDENT RELAXATION OF RAT JUGULAR VEIN

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An 'atypical' 5-HT receptor which is located on the endothelium of rat jugular vein has been characterised as 5-HT$_{2C}$-like (Bodelsson *et al.*, 1992). In the present study we have evaluated further the nature of this receptor using the recently described 5-HT$_{2B}$/5-HT$_{2C}$ selective ligand SB 200646 (*see* Baxter *et al.*, 1994) and other ligands which permit discrimination of rodent 5-HT$_2$ receptor subtypes *in vitro*.

Rat jugular vein was set up as described by Bodelsson *et al.*, (1992). Tissues were obtained from male Sprague Dawley rats (250-300g) and suspended for isometric tension recording in oxygenated (95% O_2/5% CO_2) Kreb's solution at 37°C. The Kreb's solution contained indomethacin (3.0 μM) and ketanserin (0.1 μM) to preclude involvment of prostanoids or 5-HT$_{2A}$ receptors. Rat stomach fundus was set up according to Baxter *et al.*, (1994). Two agonist concentration-effect curves were constructed in each preparation. The effects of antagonists were evaluated after an equilibration period of one hour.

In veins, pre-contracted with the stable thromboxane mimetic U-46619 (0.1 μM), the cumulative addition of 5-HT (0.1nM - 0.3 μM) yielded monophasic relaxant concentration effect curves. On repetition, concentration-effect curves to 5-HT were highly reproducible. pEC_{50} [95% C.L.] estimates of 1st and 2nd curve were 8.2 [8.0-8.3] and 8.3 [8.1-8.5] n=12, respectively. SB 200646 (0.1 - 1.0 μM) acted as a surmountable antagonist of 5-HT (pA_2 [slope] = 7.2 [1.05], $n \leq 3$). Yohimbine (0.03, 0.1 & 0.3 μM), which shows selectivity for 5-HT$_{2B}$ over 5-HT$_{2C}$ receptors (*see* Baxter *et al.*, 1994) was also a potent antagonist of 5-HT (Mean concentration-ratio ± s.e.m. = 1.6 ± 0.5, 14.9 ± 5 & 41.3 ± 13, apparent pA_2 [slope] = 7.8 [0.84]), but caused a depression in maximum response of up to 27 ± 7 %. Neither antagonist (at 0.3 μM) significantly affected endothelium dependent relaxations induced by carbachol. BW 723C86, an agonist which shows some selectivity for 5-HT$_2$-like receptors in rabbit jugular vein (Martin *et al.*, 1993 & personal communication) acted as a potent partial agonist at putative 5-HT$_{2B}$ receptors in rat stomach fundus (mean pEC_{50} [95% C.L.] & intrinsic activity ± s.e.m. = 8.0 [7.6-8.3] & 0.7 ± 0.1, n=6) but not 5-HT$_{2A}$ receptors in rat caudal artery ($pEC_{50} < 6.0$, n=4, G. S. Baxter unpublished observations). BW 723C86 also acted as a potent partial agonist with respect to 5-HT in rat jugular vein (pEC_{50} [95% C.L.] = 7.9 [7.7-8.1], intrinsic activity ± s.e.m. = 0.84 ± 0.04, n=8).

The data obtained in the present study are consistent with the presence of 5-HT$_{2B}$, and not 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors mediating relaxation of rat jugular vein. The potent agonism displayed by BW 723C86 at putative 5-HT$_{2B}$ receptors in both rat jugular vein and rat stomach fundus suggests that the 'atypical' 5-HT$_2$-like receptors in other species (Martin *et al.*, 1993) may represent species homologues of the rat 5-HT$_{2B}$ receptor.

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478P THE ACTIONS OF KETANSERIN AND RITANSERIN ON CONTRACTILE RESPONSES TO SUMATRIPTAN IN RABBIT BASILAR ARTERY

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Human 5-HT_{1D} receptors have been sub-divided into 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ subtypes (Weinshank *et al.*, 1991). Pharmacologically these two subtypes are very similar, however both ketanserin and ritanserin may permit some discrimination as they possess a 20- and 70-fold higher affinity for the 5-$HT_{1D\alpha}$ versus the 5-$HT_{1D\beta}$ subtype, respectively (*see* Kaumann *et al* ., 1993). In the present study, we have evaluated the actions of both ketanserin and ritanserin at 5-HT_1-like receptors in rabbit basilar artery (RBA).

Arteries were obtained from New Zealand White rabbits (male, 2-3 kg). Endothelial denuded ring segments (5 mm length) were suspended for isometric tension recording in oxygenated (95% O_2/5% CO_2) Kreb's solution at 37°C. All experiments were conducted in the presence of indomethacin (3 μM), cocaine (3 μM) and after tissues had been exposed to pargyline (100 μM) for 15 min followed by washout. Two cumulative concentration-effect curves to sumatriptan were constructed, at least one hour apart, in each preparation. The actions of antagonists were evaluated after an equilibration period of one hour.

Sumatriptan (0.01 - 1 μM) evoked reproducible, contractile concentration-effect curves in RBA. In most tissues, concentration-effect curves were monophasic (mean pEC_{50} [95% C.L.] = 7.1 [6.8-7.4], n = 9). However, in a few preparations a second, low potency phase of the sumatriptan concentration-effect curve was evident at concentrations of 1 μM and above. Ketanserin (0.3 - 10 μM) caused concentration-dependent, rightward displacement of concentration-effect curves to sumatriptan (mean concentration ratios ± s.e.m. = 6.0 ± 0.9, 14.2 ± 1.4, 14.5 ± 4 & 38.0 ± 6, n ≥ 4, respectively) without significant effects on the maximum response. Schild regression analysis of all data yielded an apparent pA_2 of 8.3 with slope of 0.46, indicating deviation from simple competition. Schild regression analysis of data obtained at the lower concentrations (0.3 & 1 μM) yielded an apparent pA_2 of 7.3 (slope = 0.83). Ritanserin (0.1, 0.3 & 1 μM) also acted as an antagonist of sumatriptan in RBA (mean concentration ratios ± s.e.m. = 5.8 ± 1.2, 16.8 ± 2.7 & 44.3 ± 19, n≥ 4, respectively) but caused a depression in maximum response of up to 30%. Concentration-effect curves for sumatriptan in the presence of spiperone (1.0 μM, n=6), were not significantly different from controls.

The observed deviation from simple competition with both ketanserin and ritanserin may reflect either an action of sumatriptan at multiple recognition sites or an allotopic action of these antagonists. The apparent pA_2 value determined at the lower concentrations of ketanserin in RBA are similar to the binding affinity of this ligand at 5-$HT_{1D\alpha}$ receptors (approximately 7.1) and thus, indicates some pharmacological similarity to the human 5-$HT_{1D\alpha}$ receptor subtype.

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479P PROFOUND REDUCTION IN PLATELET RESPONSES AFTER COMBINED ADMINISTRATION OF 5-HT_2 AND THROMBOXANE ANTAGONISTS

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In previous experiments in anaesthetized rats subject to abrupt reperfusion after 5 min of coronary artery occlusion, we found that combined administration of a 5-HT_2 antagonist and a thromboxane antagonist had significant antiarrhythmic activity, whereas the effects of either drug administered alone did not reach statistical significance (Shaw & Coker, 1992). We have now performed additional experiments to investigate whether alterations in platelet aggregation contribute to the antiarrhythmic effects of combined drug administration. Male Wistar rats (315 to 415 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.) and cannulae were placed in a carotid artery for blood pressure measurement and a femoral vein for drug administration. A thoracotomy was also performed to mimic the surgical preparation in our previous experiments on arrhythmias. Rats were allocated randomly to one of four groups (n=6 per group): control, 5-HT_2 antagonist - ICI 170,809 (Blackburn *et al.*, 1988), thromboxane antagonist - ICI 192,605 (Jessup *et al.*, 1988) or both drugs. Each rat received a bolus dose of ICI 170,809, 1 mg kg^{-1} (or its vehicle, acidified water) followed immediately by a continuous infusion of ICI 192,605, 1 mg kg^{-1} min^{-1} (or its vehicle, alkaline saline). Fifteen min after commencing drug administration blood was removed via the arterial cannula and placed in a plastic tube containing 50 units of heparin per ml of blood. Aliquots of whole blood (450 μl) were diluted with 550 μl of saline, incubated at 37°C, and platelet aggregation in response to ADP (10^{-7}M) or collagen (1 μg ml^{-1}), alone or in the presence of 5-HT (10^{-5}M) and/or U46619 (10^{-6}M) was measured by the impedance method (Cardinal & Flower, 1980) using a Chronolog whole blood aggregometer.

Table 1.

	ADP-induced Platelet Aggregation (ohms)				*Collagen-induced Platelet Aggregation (ohms)*			
	alone	*+5-HT*	*+U4*	*+5-HT+U4*	*alone*	*+5-HT*	*+U4*	*+5-HT+U4*
Control	4.3±0.8	6.5±1.0	14.0±2.3	16.9±2.4	3.6±0.9	10.9±1.2	11.2±2.2	14.2±1.7
ICI 170,809	4.4±1.0	4.3±1.0	13.5±3.6	12.0±3.4	4.0±0.5	5.0±0.8*	11.2±3.1	14.0±3.1
ICI 192,605	2.4±0.8	3.7±0.6	3.3±1.7*	7.5±3.7*	3.1±1.0	8.7±2.3	6.8±2.5*	8.1±2.3
Both drugs	1.2±0.3*	3.0±0.7	0.9±0.4**	1.3±0.5**	3.4±1.1	4.6±1.5**	2.9±0.6**	2.7±0.6**

*P<0.05, **P<0.01 compared with corresponding value in the control group, Kruskal-Wallis test.

The abilities of 5-HT and U44619, to potentiate platelet aggregation induced by ADP or collagen, were attenuated in blood taken from rats which had received ICI 170,809 and ICI 192,605 respectively. However, platelet responsiveness was markedly reduced in blood from rats which had received both drugs. These results indicate that in the presence of both 5-HT and thromboxane A_2, platelet aggregation is enhanced greatly, and that prevention of this interaction may account for the significant antiarrhythmic activity which was only observed when both the 5-HT antagonist and the thromboxane antagonist were administered together.

This work was supported by the British Heart Foundation.

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480P EFFECTS OF THE PUTATIVE METABOTROPIC GLUTAMATE RECEPTOR ANTAGONIST L-2-AMINO-3-PHOSPHONOPROPIONATE ON PHOSPHOINOSITIDE TURNOVER IN NEONATAL RAT CEREBRAL CORTEX

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L-2-Amino-3-phosphonopropionate (L-AP3), a putative antagonist of metabotropic glutamate receptor (mGluR) activation, has been widely used in studies investigating mGluR function in the CNS (Schoepp & Conn, 1993). However, although antagonist actions of L-AP3 have been widely reported, other effects, most notably agonist activity, have also been observed (Lonart et al., 1992). Here, we have investigated the ability of L-AP3 to inhibit 1-aminocyclopentane-1S,3R-dicarboxylate (ACPD)-stimulated inositol phospholipid hydrolysis and also whether this agent per se can cause phosphoinositide turnover in neonatal rat cerebral cortex slices.

Slices (350 x 350 μm) were prepared from 7-8 day old rats of either sex. Following pre-incubation in Krebs-Henseleit buffer (KHB) for 60 min, 25 μl packed slices were transferred to 250 μl KHB and incubated for 60 min in the presence (for total [^{3}H]inositol phosphate ([^{3}H]InsP)) or absence (for Ins(1,4,5)P_3 mass) of [^{3}H]inositol (0.5 μCi/vial). Where used as an antagonist, L-AP3 was added 15 min prior to ACPD or carbachol (CCH) challenge for a further 15 min. In addition, [^{3}H]InsP (in the presence of 5 mM LiCl) and Ins(1,4,5)P_3 accumulations in response to L-AP3 per se were also assessed. Incubations were terminated, processed and analysed for [^{3}H]InsP or Ins(1,4,5)P_3 accumulations, or [^{3}H]inositol incorporation into the cellular inositol phospholipid pool as described previously (Challiss et al., 1994).

L-AP3 (300 μM) did not significantly affect basal [^{3}H]InsP accumulation (control, 4156 $\pm$ 371; +L-AP3, 5229 $\pm$ 405 d.p.m./25 μl slices (n=4), but caused a 50% inhibition of [^{3}H]InsP accumulation stimulated by 300 μM ACPD (+ACPD, 22998 $\pm$ 377; +L-AP3/ACPD, 14992 $\pm$ 405). Following ACPD (300 μM) $\pm$ L-AP3 (300 μM) challenge, no significant differences in [^{3}H]inositol labelling of any of the inositol phospholipid species were seen. At a concentration of 1 mM L-AP3 caused a large [^{3}H]InsP accumulation whilst a similar concentration of the D-AP3 was without effect (+L-AP3, 33050 $\pm$ 2156; +D-AP3, 4840 $\pm$ 391 d.p.m./25 μl slices). Co-addition of ACPD (300 μM and L-AP3 (1 mM) resulted in a less than additive accumulation of [^{3}H]InsP. ACPD caused a large increase in Ins(1,4,5)P_3 accumulation at 15 sec (control, 22.4 $\pm$ 0.8; +ACPD (300 μM), 125.8 $\pm$ 5.7 pmol/mg protein (n=4)) and this response declined rapidly (<5 min) to a plateau about 50% above basal. L-AP3 (1 mM), but not D-AP3, caused a much slower increase in Ins(1,4,5)P_3 accumulation which only approached a maximal value (97.0 $\pm$ 3.3 pmol/mg protein) 5-10 min after L-AP3 addition.

Thus, L-AP3, at concentrations up to 300 μM, inhibited ACPD-stimulated phosphoinositide turnover in neonatal rat cerebral cortex slices. However, at higher concentrations, L-AP3 stimulated both [^{3}H]InsP accumulation and Ins(1,4,5)P_3 mass accumulation. Irrespective of whether this latter stimulatory action of L-AP3 proves to be mediated via mGluRs, these results suggest that caution should be exercised in the interpretation of studies which employ this agent as an antagonist of mGluR-mediated phosphoinositide turnover.

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481P EFFECTS OF CHRONIC ETHANOL TREATMENT ON AMPA BINDING SITES IN CEREBRAL CORTEX

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After chronic ethanol ingestion, behavioural hyperexcitability is seen, and the number of NMDA receptor-ion channel complexes in CNS is increased (Hoffman et al., 1990). AMPA receptors, however, have been little studied. In this work we investigated the state of the AMPA receptors after chronic ethanol treatment.

Male rats, Wistar strain, 150-200g, were given ethanol as a liquid diet, with 3.5% v/v ethanol for two days, and 7% v/v ethanol for a further ten days. Control rats received isocalorific control diet. Brains were removed and dissected at appropriate times and the tissues frozen at -25°C. Cortical membranes were prepared 0, 7 and 24h from cessation of chronic ethanol treatment. One measurement of binding was then made at each withdrawal time, plus controls, per experimental day. Tissues were homogenised in Tris buffer and [^{3}H]-AMPA binding measured using the method of Honoré and Drejer (1988) with 1 mM glutamate to estimate nonspecific binding. Incubation was terminated after 45 min by rapid filtration through Whatman GF/B filters. Results were calculated by Scatchard analysis after scintillation counting.

Specific [^{3}H]-AMPA binding in the presence of SCN^- ions gave a curvilinear Scatchard plot with two binding sites (B_{max1} and B_{max2}) with the following values (means $\pm$ s.e.m., n values were 5 per treatment group):

Treatment	B_{max1} (fmol/mg protein)	K_{d1} (nM)	B_{max2} (fmol/mg protein)	K_{d2} (nM)
Controls	47 ± 11	44 ± 5	155 ± 55	298 ± 34
Ethanol, 0h withdrawal	59 ± 18	46 ± 6	192 ± 43	312 ± 57
Ethanol, 7h withdrawal	53 ± 12	49 ± 16	185 ± 39	305 ± 73
Ethanol, 24h withdrawal	45 ± 13	51 ± 13	149 ± 65	309 ± 85

After chronic ethanol treatment the mean Bmax values were increased, but the differences were not significant ($P > 0.05$, compared with controls, Student's t-test). However, when the results obtained on each experimental day were compared, the values obtained at 0h withdrawal were always higher than control values ($P < 0.05$, sign test). Similar patterns of results were obtained for the high and low affinity receptor binding sites. There were no changes in Kd values.

The results indicate that chronic ethanol treatment may cause some upregulation of both high and low affinity [^{3}H]-AMPA binding sites, at 0h and 7h, but not at 24h, from withdrawal. Behavioural hyperexcitability in this model is seen at 7h withdrawal but has disappeared by 24h into the withdrawal period; alcohol would be still present in the CNS at the 0h interval.

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482P INTRACEREBRAL MICRODIALYSIS COMBINED WITH RECORDING OF EXTRACELLULAR FIELD POTENTIAL: A NOVEL METHOD FOR THE INVESTIGATION OF DEPOLARISING DRUGS *IN VIVO*

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The grease-gap preparation is a useful method for studying *in vitro* the pharmacology of excitatory amino acids (Harrison & Simmonds, 1985). It is based on the quantitative recording of depolarisations evoked by excitatory agonists in brain slices. The purpose of this study was to examine whether similar responses can be recorded *in vivo*, using a recording electrode incorporated into a microdialysis probe.

Rats were anaesthetised throughout the experiments with halothane (0.8-2% in N_2O:O_2 70:30). Microdialysis probes (2 mm membrane length; Obrenovitch *et al.*, 1993) were implanted in the striatum and perfused with artificial CSF (ACSF; composition in mM: NaCl, 125; KCl, 2.5; $MgCl_2$, 1.18; $CaCl_2$, 1.26; KH_2PO_4, 2) at 1 µl/min. Extracellular field potential (EFP) was recorded between the microdialysis electrode and a reference beneath the scalp. After a 2 h control period, increasing concentrations of N-methyl-D-aspartate (NMDA) were applied for 2 min (25, 100, 250 and 500 µM in ACSF, pH adjusted to 7.3), each followed by 30 min of perfusion with normal ACSF. This sequence was repeated with Mg-free ACSF. Finally, the non-competitive NMDA antagonist MK-801 was administered (2 mg/kg, i.p.) and the 500 µM NMDA application in Mg-free ACSF repeated.

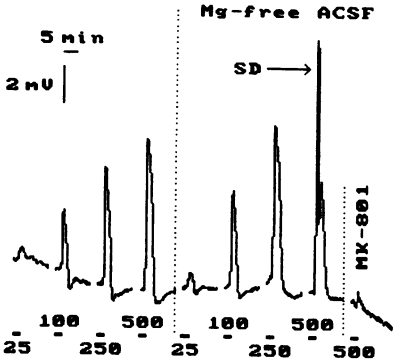


Fig. 1: Dose-dependent responses evoked by NMDA *in vivo*.

NMDA applied to the striatum evoked a dose-dependent depolarisation, followed by hyperpolarisation (Figure 1). This pattern is very similar to that produced in the cortical wedge preparation (Harrison & Simmonds, 1985). Occasionally, high doses of NMDA produced a rapid and transient further depolarisation indicating initiation of spreading depression (Figure 1, SD). The response evoked by 500 µM of NMDA was markedly reduced by MK-801 [9.7 ± 1.4 (n=5) vs. 3.2 ± 1.2 (n=7) mV; mean ± s.e.mean; $p<0.001$]. In some experiments, NMDA-evoked responses were slightly higher with Mg-free ACSF (Figure 1), but this difference was not significant.

Conclusion: Microdialysis combined with EFP recording provides quantitative information on the effects of glutamatergic drugs *in vivo*. Reversible alterations of the ionic composition of the ACSF and addition of specific drugs, may contribute to a better understanding of the function and control of glutamate receptors in distinct brain regions.

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483P AMPA RECEPTOR STIMULATION INCREASES 5-HT RELEASE IN RAT STRIATUM AND HIPPOCAMPUS *IN VIVO*

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Recent evidence has suggested that release of 5-hydroxytryptamine (5-HT) within the CNS may be under glutamatergic control (Bequet et al., 1990). Using push-pull cannulae these authors observed that infusion of N-methyl-D-aspartate (NMDA) into cat caudate decreased 5-HT release. In the present study we have investigated the effects of stimulating the α-amino-3-hydroxy-5-methyl-4-isozoxazole-propionate (AMPA) glutamate receptor subtype, on dialysate concentration of 5-HT in rat hippocampus and striatum.

Male Wistar rats (280-350g) were implanted, under chloral hydrate anaesthesia (400mg/kg), with concentric dialysis probes into either the anterior corpus striatum or ventral hippocampus. The following day rats were dialysed with artificial cerebrospinal fluid (0.5 µl/min) containing 1µM citalopram. Four 30 min samples were collected prior to infusion of AMPA alone, or in combination with either the non-NMDA glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3,-dione (CNQX; 200 µM) or tetrodotoxin (TTX; 1 µM). 5-HT concentration was determined using HPLC with electrochemical detection. Basal dialysate concentration of 5-HT was 104 ± 12 and 53 ± 8 fmol/10µl in hippocampus and striatum respectively (n = 40).

AMPA (50-500 µM) caused a concentration-dependent increase (180-584 % change, n = 6-8) in striatal dialysate 5-HT. When 100 µM AMPA was infused in the presence of CNQX the effect of AMPA on dialysate 5-HT was completely antagonised. TTX significantly decreased basal 5-HT for 3 h post infusion (maximal decrease 15 ± 5 % of basal, n =5). In the presence of TTX, 100 µM AMPA failed to increase dialysate 5-HT, which fell in a very similar manner to that seen with TTX alone.
In the hippocampus 5-HT release was much more sensitive to AMPA stimulation. At 1 and 10 µM AMPA, 5-HT release was increased, by up to 243 ± 14 and 533 ± 40 % of basal respectively (mean ± s.e.mean, n = 6-8). At the higher concentration 5-HT levels were significantly elevated for over 2 h. 50 µM AMPA increased 5-HT release to a similar extent to that seen with 10 µM AMPA, but the duration was only about 1 h. When 100 µM AMPA was infused dialysate 5-HT was significantly decreased to around 50 % of basal for 3 h. However, if dialysates were collected every 5 min, a brief increase in 5-HT (5-10 min duration) could be seen. This may reflect desensitization of the hippocampal AMPA receptor (cf. Mayer and Vyklicky, 1989). When co-infused with 10 µM AMPA, CNQX and TTX both modified the agonist response in a manner very similar to that seen in the striatum. These data suggest that the effect of AMPA on 5-HT release is receptor mediated and voltage-dependent in hippocampus and corpus striatum, although distinct profiles were observed for the two brain structures.

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484P INTERLEUKIN-1 RECEPTOR ANTAGONIST INHIBITS NMDA AND AMPA RECEPTOR-INDUCED BRAIN DAMAGE IN THE RAT

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The cytokine interleukin-1β (IL-1β) is synthesized in the brain in response to injury and has been proposed as a mediator of excitotoxic damage. Interleukin-1 receptor antagonist (IL-1ra) is an endogenous antagonist of IL-1 which inhibits excitotoxic damage due to over-activation of the N-methyl-D-aspartate (NMDA) receptor (Relton & Rothwell, 1992). Excitotoxic neuronal damage can also be induced by over-activation of the non-NMDA receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate (Meldrum & Garthwaite, 1990). The aim of this study was to investigate the involvement of IL-1β in excitotoxic brain damage induced by over-activation of NMDA or AMPA receptors.

Male, Sprague Dawley rats (250-350g) were anaesthetized and placed in a stereotaxic frame. Striatal lesions were induced by local infusion of 1μl cis-2,4-methanoglutamate (MGlu, 10nmol) or S-AMPA (10-20nmol), activating NMDA and AMPA receptors respectively. Animals were sacrificed 24h or 48h later and lesion volume assessed on serial 250μm striatal coronal sections stained with tetrazolium. All results are expressed as the mean$\pm$s.e.mean.

Intrastriatal infusion of 10nmol MGlu resulted in reproducible lesions (mean volume 24.77$\pm$2.25mm^3, n=5). Several doses of S-AMPA (10-20nmol) were tested and damage was assessed in separate groups of animals killed 24 or 48h after infusions. A dose of 15nmol was chosen for further studies and measurements were made 48h after infusion as this produced optimal damage, which was limited to the striatum. Infusion of 1μl of vehicle, phosphate buffered saline (PBS) or IL-1ra (Synergen, USA) alone did not cause neuronal damage. Pretreatment with MK-801 (4mg/kg, i.p, 30min prior to infusion) resulted in a marked and significant reduction (69%) in the size of the lesion induced by MGlu when compared to vehicle (0.25ml PBS) injection (Vehicle; 22.63$\pm$1.54mm^3, n=11 versus MK-801; 7.05$\pm$2.19mm^3, n=11, $P<0.001$). Whereas MK-801 pretreatment had no effect on S-AMPA induced damage. In comparison, co-infusion of IL-1ra (5μg in 1μl) with 10nmol MGlu resulted in a 46% reduction in lesion volume (Vehicle; 23.26$\pm$1.19mm^3, n=8 versus IL-1ra; 12.47$\pm$2.61mm^3, n=10, $P<0.01$). Additionally IL-1ra produced a 43% inhibition of 15nmol S-AMPA mediated damage (Vehicle; 12.78$\pm$1.42mm^3, n=12 versus IL-1ra; 7.26$\pm$1.78mm^3, n=12, $P<0.05$). However co-infusion of IL-1β (100U) with MGlu (5nmol) did not increase striatal damage. In separate studies, infusion of IL-1β (100U) alone did not cause any detectable neuronal damage when assessed by haematoxylin and eosin staining.

The data described above provide evidence for the role of endogenous IL-1 in excitotoxic damage induced by activation of both NMDA and non-NMDA receptors, although direct effects of IL-1β in the absence of excitotoxins remain to be clarified.

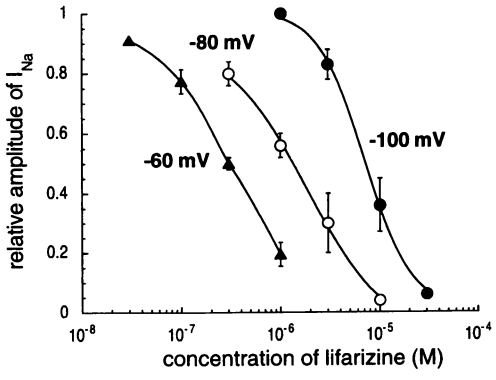
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485P THE NEUROPROTECTIVE AGENT, LIFARIZINE (RS-87476), INHIBITS SODIUM CURRENTS VIA AN INTERACTION WITH THE SLOWLY INACTIVATING STATE OF THE CHANNEL

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Lifarizine (RS-87476) is neuroprotective in animal models of both focal (Kucharczyk *et al.*, 1991) and global ischaemia (Alps *et al.*, 1990). Various lines of evidence support the contention that the drug may work via an interaction with voltage-activated Na^+ channels: lifarizine displaces [^{3}H]-batrachotoxin from rat brain synaptosomes (IC_{50} = 55 nM; C.M. Brown, Syntex Research, *unpublished data*) and inhibits Na^+ currents in N1E-115 cells by an interaction with the inactivated state(s) of the channel (Sheridan *et al.*, 1991). As there are at least 2 kinetically distinguishable states of inactivation of the Na^+ channel (*fast* and *slow*), the present experiments were designed to establish which of these is important for expression of the inhibitory action of lifarizine on whole-cell Na^+ currents measured in voltage clamped N1E-115 neuroblastoma cells.

To study the effect of lifarizine on *fast inactivation*, steady-state inactivation curves were constructed using 1 s pre-pulses at membrane potentials ranging from -100 to -10 mV in cells before and after treatment with lifarizine (1 µM, 10 min). Using this protocol, lifarizine affected neither the potential at which 50 % of the channels were inactivated (V_{half}: control -64.2 ± 3.7 mV, lifarizine -66.0 ± 3.9 mV; $P > 0.05$, n = 2) nor the slope of the curve (*k*: control 6.8 ± 0.5 mV/*e*-fold, lifarizine 7.0 ± 0.4 mV/*e*-fold; $P > 0.05$, n = 2). *Slow inactivation* was studied by clamping the cells at potentials ranging from -100 to -50 mV, holding at each step for at least 1 min. Under these conditions 1 µM lifarizine induced a negative shift of the steady-state inactivation curve (V_{half}: control -71.8 ± 1.6 mV, lifarizine -81.1 ± 1.4 mV; $P < 0.001$, n = 4) without affecting the slope (*k*: control 6.2 ± 0.3 mV/*e*-fold, lifarizine 6.4 ± 0.1 mV; $P > 0.05$, n = 4). In the final series of experiments, the inhibitory effect of lifarizine on I_{Na} was examined on cells clamped at 3 different holding potentials: -100, -80 or -60 mV. Current/voltage curves were obtained before and 10 min after application of lifarizine and the effect of the drug on the peak current was measured. The graph (*right*) shows that the potency of lifarizine was enhanced at the more depolarized holding potentials (IC_{50} = 7.3, 1.3 and 0.3 µM at -100, -80 and -60 mV, respectively).



The present data demonstrate that lifarizine interacts predominantly with the slowly inactivating state of the Na^+ channel. The enhancement of the potency of lifarizine at depolarized potentials is consistent with this interaction.

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486P THE ACTIONS OF BOSENTAN, AN ENDOTHELIN RECEPTOR ANTAGONIST, ON CEREBRAL ARTERIOLES *IN SITU*

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Endothelin has been implicated in a variety of cerebrovascular pathologies. However, the lack of brain penetrating endothelin antagonists has hindered the elucidation of the role of endothelins in cerebrovascular disease. In this study we assessed the effects of the endothelin receptor antagonist, 4-tert-Butyl-N-[6-(2-hydroxy-phenoxy)-2,2'-bipyrimidin-4-yl]-benzenesulfonamide (Bosentan) (Clozel et al., 1993) on cerebral resistance vessels and examined its ability to antagonise the effects of exogenous endothelin-1 (ET-1) after both topical and systemic administration. *In vitro*, Bosentan has been shown to be selective for the ET_A and ET_B receptor subtypes (Clozel et al., 1993).

Anaesthesia in adult cats was induced with alphaxolone/alphadolone (Saffan, Glaxo) and maintained using α-chloralose. A left parietal craniectomy was performed, the surface of the brain exposed and the calibre of the pial arterioles lying on the surface of the cortex were measured using an image splitting technique (Robinson & McCulloch, 1990). ET-1 (10nM), artificial cerebrospinal fluid (CSF) and Bosentan were applied perivascularly into the subarachnoid space surrounding the vessels. ET-1 (10nM) and Bosentan were co-administered in a similar manner. Bosentan (17μmol kg^{-1}) was administered intravenously and ET-1 injections into the subarachnoid space were carried out. All results are expressed as mean ± s.e. mean (percentage change from baseline), n=6-13 arterioles in each group. Statistical analysis was carried out using ANOVA. ††P<0.01 (from CSF); **P<0.01 (from ET-1).

Application of CSF resulted in a minimal effect on pial arteriolar calibre. Perivascular microapplication of Bosentan (0.3, 3, 30, 300μM) had minimal effects on pial arteriolar calibre. ET-1 application effected a vasoconstrictor response and co-injection with Bosentan resulted in a concentration dependent attenuation of the ET-1 constriction (Table 1). Intravenous administration of Bosentan (17μmol kg^{-1}) resulted in an inhibition of the ET-1 response (-1.64 ± 0.9% after drug administration compared to -39.2 ± 2.7% prior to intravenous administration; P<0.01).

TABLE 1

Perivascular Microinjection	Pial Arteriolar Calibre (% Change from Baseline)	Number of Arterioles Examined
CSF	+0.9 ± 1.7	11
ET-1 (10nM)	-39.2 ± 2.7 ††	13
ET-1 (10nM) + Bosentan (0.3μM)	-27.5 ± 5.9	7
ET-1 (10nM) + Bosentan (3μM)	-13.1 ± 3.3 **	8
ET-1 (10nM) + Bosentan (30μM)	-3.7 ± 2 **	7

These results show that Bosentan is a potent endothelin antagonist and biologically active amounts penetrate the blood-brain barrier to antagonise the effects of perivascularly applied endothelin. Bosentan would appear to be a useful tool in elucidating of the role of the endothelins in the cerebral circulation.

TRP is supported by an MRC/CASE studentship. Bosentan was a gift from F. Hoffman La Roche Ltd., Switzerland.

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487P A NEUROCHEMICAL STUDY OF NON-ALZHEIMER DEMENTIA

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Preliminary studies of non-Alzheimer dementias due to lobar atrophy indicate that these disorders have a neurochemical profile distinct from Alzheimer's disease (AD: Francis *et al.*, 1993). To further these studies, choline acetyltransferase (ChAT) activity and binding to $5HT_{1A}$ (2nM 2-(N,N-di[2,3(n)-^{3}H]propylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene, 8OH-DPAT) and $5HT_{2A}$ (2nM [^{3}H]-Ketanserin) receptors and somatostatin-like immunoreactivity (SLIR) were determined as described previously, (Bowen *et al.*, 1989) in frontal (Brodmann Area, BA 9) and temporal (BA 21) cortex of 49 previously frozen human brains. The brains were matched for age and *post mortem* delay and were from either control subjects (free of gross neurological or psychiatric disorders) or patients suffering from Pick's disease, Dementia of Frontal Lobe Type (DFT) or AD.

Table 1: Neurotransmitter markers in Pick's disease, DFT and AD. Values are mean ± s.e. mean. * significantly different from control, one-way ANOVA followed by Dunnett's t-test, $p < 0.05$.

	ChAT pmoles ACh/mg prot/min		$5HT_{1A}$ fmoles/mg prot		$5HT_{2A}$ fmoles/mg prot		SLIR fmoles/mg prot	
	BA 9	BA 21	BA 9	BA 21	BA 9	BA 21	BA 9	BA 21
Control (24)	104 ± 11	151 ± 11	10 ± 1	28 ± 2	21 ± 1	40 ± 3	148 ± 16	79 ± 11
Pick's (10)	90 ± 14	116 ± 18	5 ± 1*	11 ± 4*	9 ± 2*	19 ± 4*	207 ± 25	64 ± 24
DFT (6)	94 ± 19	138 ± 28	8 ± 1	18 ± 5	10 ± 2*	22 ± 6*	254 ± 35*	108 ± 11
AD (9)	47 ± 9*	77 ± 20*	8 ± 1	22 ± 4	11 ± 2*	25 ± 2*	154 ± 35	47 ± 16

The main finding of this study (Table 1) was that ChAT activity in patients with Pick's disease and DFT was comparable with that of controls rather than reduced as in AD (data above) and Lewy body dementia (Perry *et al.*, 1990). The lack of reduction in SLIR concentration may indicate sparing of at least a subpopulation of cortical interneurones in all three disorders. Reduced binding to the $5HT_{2A}$ receptor in AD has been reported to be associated with the presence of abnormal non-cognitive behaviour (Procter *et al.*, 1992). It is therefore of interest that such behavioural abnormalities are also present in the clinical syndromes of Pick's disease and DFT. The reason for the reduction in binding to $5HT_{1A}$ receptors in only Pick's disease is unknown at present.

In summary, the present study indicates that dementias associated with lobar atrophy have neurochemical profiles distinct from AD. Further studies are required to improve the understanding of the relationship between these conditions.

Supported by the Wellcome Trust.

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488P MICRODIALYSIS STUDY INVESTIGATING THE EFFECTS OF GR127935, A POTENT 5-HT$_{1D}$ RECEPTOR ANTAGONIST, ON CORTICAL LEVELS OF 5-HT IN THE GUINEA-PIG

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GR127935, (N-[4-Methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1,-biphenyl]-4-carboxamide) (Skingle et al., 1993) is a potent 5-HT$_{1D}$ receptor antagonist at somatodendritic 5-HT$_{1D}$ autoreceptors in the dorsal raphe of the guinea-pig (Starkey & Skingle,1994). The effects of GR127935 on 5-HT efflux has now been studied using intracerebral microdialysis.

Male Dunkin Hartley guinea-pigs (Interfauna; 250-350 g) were anaesthetised with chloral hydrate (400 mg/kg i.p.) for stereotaxic surgery. Dialysis loops were prepared (Sleight et al, 1988) and implanted into the frontal cortex (co-ordinates from the intra aural line: rostral-caudal: +16 mm; sagittal: +2 mm; vertical from the dura -1.5 mm). Anaesthesia was maintained with chloral hydrate throughout the experiment. Two hours after implantation, 4 x 20 min samples were taken to measure basal extracellular levels of 5-HT. The loops were perfused with artifical CSF (aCSF) at a rate of 2 µl/min. The dialysis samples were analysed for 5-HT using high performance liquid chromatography with electrochemical detection . At least 4 animals were used in each treatment group and significance was taken at the $p<0.05$ level (students unpaired t-test).

Basal extracellular levels of 5-HT in the guinea-pig frontal cortex in placebo-treated animals were 30 ± 4 fmoles / 20 µl perfusate (limit of sensitivity for assay: 5 fmoles/20 µl). When GR127935 (100 nM) was infused through the dialysis probe into frontal cortex it caused a significant increase (61±8%) in cortical extracellular levels of 5-HT. The increase was transient (≅ 40 min) even in the presence of a constant infusion of the antagonist. The transient increase in 5-HT levels was abolished by tetrodotoxin (1µM). When guinea-pigs pretreated with saline (0.9%) were given the 5-HT$_1$ receptor agonist GR46611, 10 mg/kg s.c., (Skingle et al, 1994) a significant and sustained (>100 min) reduction in extracellular levels of 5-HT (65±5%) was seen. This response was abolished in animals pretreated with GR127935, 0.05 mg/kg i.p (1h ptt.). Paradoxically, systemic administration of higher doses of GR127935, 0.1 - 1 mg/kg i.p., in naive animals caused significant and sustained (>120 min) decreases (>65%) in cortical levels of 5-HT.

The increase in extracellular 5-HT seen following infusion of GR127935 into frontal cortex may be due to GR127935 blocking 5-HT terminal autoreceptors causing a subsequent increase in the outflow of 5-HT from presynaptic terminals. This conclusion is supported by the ability of GR127935 to block the decrease in 5-HT induced by the 5-HT$_1$ receptor agonist GR46611. The mechanism of the sustained decrease in 5-HT following systemic administration of GR127935 is unknown. However it is possible that GR127935 acts at several sites within the CNS and the net overall effect is to decrease 5-HT levels in terminal regions. For example, it is possible that GR127935 increases 5-HT levels in the raphe region causing a decrease in cell firing and a subsequent decrease in 5-HT in terminal-containing regions.

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489P EFFECT OF THE SELECTIVE 5-HT$_{1D}$ RECEPTOR ANTAGONIST, GR 127935, ON *IN VIVO* 5-HT RELEASE, SYNTHESIS AND TURNOVER IN THE GUINEA-PIG FRONTAL CORTEX

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5-HT$_{1D}$ receptor binding affinity has been tentatively correlated with 5-HT terminal autoreceptor function (Hoyer & Middlemiss, 1989). In this study we report the effect of the selective 5-HT$_{1D}$ receptor antagonist, GR127935 (Skingle *et al*, 1993), on *in vivo* 5-HT extracellular concentrations, synthesis and turnover in the guinea-pig frontal cortex.

Male Dunkin Hartley guinea-pigs (350-450g) were anaesthetised with methoxyflurane and microdialysis probes implanted in the frontal cortex (4.5mm anterior, 2mm lateral to Bregma, depth 3mm). Probes were secured and the animals allowed to recover for 24 hours. Probes were perfused with artificial CSF at a flow rate of 2µl/min and samples collected every 20 min. Animals were freely-moving with access to food and water. 5-HT synthesis was estimated from the 5-HTP accumulated after treatment with NSD1015, an inhibitor of L-aromatic amino acid decarboxylase. Vehicle or GR127935 (10-300µg/kg i.p.) were administered 50 min before NSD1015 (100mg/kg i.p.) and the animals were sacrificed after 75 min. 5-HT turnover was calculated as the 5-HIAA/5-HT ratio an hour after the animals were dosed with vehicle or GR127935 (10-300µg/kg i.p.). All samples were analysed with HPLC-ECD.

Basal levels of 5-HT in the microdialysates were 28±6fmol/40µl over a 320 min period. GR127935 (10µM), when perfused directly through the dialysis probe, increased 5-HT concentrations to a maximum of 189±50% of basal (n=4) 80 min after the drug application, with a return to basal levels on removal of the drug. However, when administered systemically, GR127935 (300µg/kg i.p.) significantly decreased 5-HT levels to a minimum of 31±12% of basal (n=6, $p<0.01$) after 140 min. GR127935 (10-3µg i.p.) increased 5-HT synthesis dose-dependently to a maximum of 233±41% of control (n=6, $p<0.05$) and increased 5-HT turnover to a maximum of 167±17% of control (n=6, $p<0.01$).

The ability of GR127935 to increase 5-HT levels on local perfusion supports the claim that the terminal autoreceptor is of the 5-HT$_{1D}$ receptor subtype. However, the unexpected decrease on systemic administration of GR127935 is difficult to explain but cannot be due to an inhibition of synthesis since this was increased. It is possible that the decrease is caused by blockade of 5-HT$_{1D}$ receptors in the raphe region (Starkey and Skingle, 1994), increasing the extracellular 5-HT and leading to 5-HT$_{1A}$ receptor-mediated inhibition of cell firing.

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490P DOES 5-HT$_{2C}$ RECEPTOR ACTIVATION CAUSE EXCITATION OF SUBSTANTIA NIGRA PARS RETICULATA NEURONES IN RAT BRAIN SLICES?

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The neurones of the substantia nigra pars reticulata (SNr) constitute a major output relay nucleus for the basal ganglia, thereby playing an important role in the extrapyramidal control of motor behaviour (Chevalier & Deniau, 1990). Innervation of this brain region by 5-hydroxytryptamine (5-HT) - containing fibres has been demonstrated (Wirtchaftler et al., 1987). We have used electrophysiological methods to establish a functional role for 5-HT in modulating the excitability of these cells, and a range of 5-HT receptor ligands to determine the receptor subtype involved.

Extracellular single unit recordings were made from SNr neurones in parasagittal slices of rat midbrain, maintained at 35°C in a continuously flowing (at 1.5 ml/min) artificial cerebrospinal fluid medium containing (mM) NaCl (126), KCl (2.5), NaH_2PO_4 (1.2), $MgCl_2$ (1.3), $CaCl_2$ (2.4), $NaHCO_3$ (26) and D-glucose (10), saturated with 95% O_2 / 5% CO_2. SNr neurones fired action potentials spontaneously at rates of 0 - 35.5 Hz (mean 9.1 $\pm$ 0.43 Hz, n = 206) with action potential waveforms of duration 0.95 $\pm$ 0.07ms (19 cells).

Application of 5-HT (0.3 - 100 μM) in the superfusate caused a concentration-dependent increase in basal firing rate of up to 233 % over basal rate in 168 of the 206 cells examined. This excitation took between 3 - 5 min to attain a maximum response and was reversible within 10 - 15 min of washout of 5-HT. The pharmacology of the 5-HT-induced excitation was assessed using 5-HT receptor antagonists, applied for at least 10 min before reapplication of 5-HT at concentrations (3-10 μM) shown to be submaximally effective on the same cell. Methysergide (0.1 μM), which would be expected to block significantly 5-HT actions at 5-HT$_{1D}$ and 5-HT$_2$ receptors (Hoyer, 1989), reduced the 5-HT excitation to 33.5 $\pm$ 12.4 % of control levels (n = 9 cells). However, spiperone (0.1 μM), which binds with high affinity to 5-HT$_{1A}$ and 5-HT $_{2A}$ receptors (Hoyer, 1989), was without effect (5-HT excitations were 100.7 $\pm$ 13.2 % of control; n = 8), as was the 5-HT$_{1A/1B}$ antagonist (Hoyer, 1989) pindolol (1 μM; 91.6 $\pm$ 16 of control; 5 cells). Additionally, the 5-HT$_2$ - preferring agonist α-methyl 5-HT (10 μM) caused a reversible increase in firing rate in all 8 cells examined (mean increase in firing rate of 58 $\pm$ 15.6% above basal).

Taken together, these results argue for the involvement of 5-HT$_{2C}$ receptors in mediating the excitation by 5-HT of SNr neurones in rat brain. However, the possible involvement of other newly identified 5-HT receptors cannot be completely discounted.

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491P ATTENUATION OF CATALEPSY BY THE 5-HT$_{1A}$/5-HT$_7$ AGONIST, 8-OH-DPAT, IS MEDIATED BY POSTSYNAPTIC 5-HT RECEPTORS

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Recently, we reported that 8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT) reduced the catalepsy induced by dopamine D2-selective antipsychotics (Needham *et al.*, 1993). However, it was not clear which population of 5-HT$_{1A}$ receptors was involved in this process, i.e. somatodendritic or postsynaptic. We have now resolved this issue by examining the anti-cataleptic response following the selective depletion of 5-HT by p-chlorophenylalanine (PCPA).

Male rats (130-190g; Charles River) received 3 days of treatment with PCPA (150 mg/kg) or vehicle i.p. Catalepsy to raclopride and its attenuation by 8-OH-DPAT (0.5 mg/kg, sc) were measured as described by Needham *et al.* (1993). 5-HT, DA and NA levels in striatum, nucleus accumbens, hypothalamus, hippocampus and frontal cortex were measured by h.p.l.c. with electrochemical detection.

Effect of PCPA treatment on the attenuation of cataleptic responses to raclopride by 8-OH-DPAT and on striatal monoamine levels

	Catalepsy (ED50; mg/kg)		Striatal monoamine levels (ng/g wet weight) 5-HT	DA	NA
Vehicle + vehicle	5.3 (2.6 - 11.0)	3 days vehicle	484.8 ± 22.5	8820.7 ± 303.2	65.0 ± 13.2
PCPA + vehicle	8.5 (3.4 - 21.4)	3 days PCPA	47.0 ± 10.0***	8682.3 ± 243.6	52.2 ± 10.1
Vehicle + 8-OH-DPAT	24.9 (7.5 - 83.0)*				
PCPA + 8-OH-DPAT	55.6 (18.8 - 163.8)*				

Raclopride ED_{50} values ± 95% confidence limits from 5 groups of 5 rats. * $p < 0.05$ versus appropriate control; Litchfield and Wilcoxon. Other data are means ± s.e.of mean; n = 6. *** $p < 0.001$ versus vehicle; Student's t-test.

PCPA treatment selectively and markedly depleted 5-HT in all brain areas. Frontal cortex and nucleus accumbens were affected least, but even these tissues had residual 5-HT which was less than 15% of control values. There was no significant loss of DA or NA. The response to 8-OH-DPAT was not attenuated by this treatment indicating that its site of action is postsynaptic, probably at 5-HT$_{1A}$ receptors, although a role for 5-HT$_7$ receptors can not be ruled out. In addition, there was a non-significant trend to a reduction in catalepsy in both PCPA treated groups which probably reflects the removal of tonic 5-hydroxytryptaminergic inhibition of DA release.

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492P *IN VITRO* AND *IN VIVO* LABELLING OF CENTRAL 5-HT$_{1A}$ RECEPTORS WITH A NEW SELECTIVE AGONIST RADIOLIGAND [^{3}H]S 14506

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Previous studies have shown that S 14506 [1[2-(4-fluoro-benzoylamino)ethyl]-4-(7-methoxynaphtyl)piperazine] is a high affinity ligand of 5-HT$_{1A}$ receptors (pKi = 9.01) with anxiolytic-like properties (Colpaert et al., 1992). Further investigations were performed to characterize its agonist/antagonist interaction at central 5-HT$_{1A}$ receptors using [^{3}H]S 14506 as radioligand.

Saturation experiments revealed a single class of [^{3}H]S 14506 high affinity binding sites (K_d = 0.15 ± 0.02 nM) in rat brain membranes. Although a highly significant correlation (r = 0.98) existed between the regional distribution of [^{3}H]S 14506 specific binding and that of the prototypical 5-HT$_{1A}$ receptor radioligand, [^{3}H]8-OH-DPAT, in the rat and the mouse brain, the B_{max} of [^{3}H]S 14506 high affinity sites was markedly higher (+88-149%) than that of [^{3}H]8-OH-DPAT specific binding sites in all regions examined. However, displacement studies with fifteen 5-HT receptor ligands confirmed that the pharmacological profile of [^{3}H]S 14506 binding sites corresponded to that of [^{3}H]8-OH-DPAT binding sites (r = 0.90). In contrast to [^{3}H]8-OH-DPAT binding, which is highly sensitive to the inhibitory effects of GppNHp (IC_{50} = 1.0 µM) and N-ethyl-maleimide (-95% at 1.0 mM), that of [^{3}H]S 14506 was resistant to these agents. These data led us to use [^{3}H]S 14506 for the possible labelling of 5-HT$_{1A}$ sites in vivo in adult male mice. One hour after the i.v. injection of 12 µCi of [^{3}H]S 14506, the regional distribution of ^{3}H was heterogeneous in brain with the highest levels in the hippocampus (70.9 ± 8.1 dpm/mg tissue) and the lowest in the cerebellum (30.1 ± 2.5 dpm/mg tissue). Pretreatment with S 14506 and other 5-HT$_{1A}$ receptor ligands (see Lanfumey et al., 1993) markedly lessened these regional differences as shown by the resulting decrease in the ratio of ^{3}H accumulated in the hippocampus to that in the cerebellum (Table 1).

Table 1: In vivo labelling of 5-HT$_{1A}$ receptors by [^{3}H]S 14506

Pretreatment	**R**
saline	2.23 ± 0.16
S 14506 (0.5 mg/kg)	1.19 ± 0.07*
(+)WAY 100135 (10 mg/kg)	1.28 ± 0.10*
S 20499 (50 mg/kg)	1.49 ± 0.06*

S 14506, (+)WAY 100135, S 20499 or saline was injected i.p. 20 min before [^{3}H]S 14506 (12 µCi i.v.), and mice were killed 60 min later. R is the ratio of ^{3}H accumulated in the hippocampus to that in the cerebellum ($n \geq 4$, $*P < 0.05$).

These data indicate that [^{3}H]S 14506 is a novel radioligand for the specific labelling of 5-HT$_{1A}$ receptors both in vitro and in vivo. It should be of interest for in vivo investigations (SPECT, PET) of central 5-HT$_{1A}$ receptors in man.

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493P CHARACTERISATION OF AN ENDOTHELIN-CONVERTING ENZYME ACTIVITY IN THE ISOLATED GALLBLADDER OF THE GUINEA-PIG

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Endothelins (ETs: ET-1, ET-2, ET-3) are potent contractors of smooth muscle. In strips of the guinea-pig gallbladder, endothelins produce contractions through ET_A and ET_B receptors (Battistini et al., 1994). Endothelin precursors (big ET-1, 2 and 3) are inactive unless they are converted by a putative endothelin-converting enzyme (ECE) into the mature peptides (Yanagisawa et al., 1988). Here we have compared the activities of the endothelins and their precursors, big ET-1 (porcine, 1-39), big ET-2 (1-38) and big ET-3 (1-41, amide) in the isolated gallbladder of the guinea-pig to assess the activity of ECE in this tissue. The responses to endothelin precursors were also characterized in the presence of several protease inhibitors.

Male DH guinea-pigs (350-450 g) were killed by cervical dislocation and the gallbladder was rapidly excised and trimmed free of connective tissue and fat. The gallbladder was opened along its longitudinal axis and two muscle strips prepared by cutting parallel to the long axis. The tissues were then mounted in 10 ml organ baths and connected to isometric transducers (Hugo Sachs Electronik KG, model K30, Germany) coupled to a chart recorder (Graphtec Corp., model WR3310, Japan). The tissues were equilibrated for 1 hour (with washing every 15 min) in gassed (95% O_2: 5% CO_2) Krebs-Henseleit solution (37°C, pH 7.4). Contractions are expressed as % of that induced by 5 µM histamine. Protease inhibitors (phosphoramidon (0.1, 1 or 3 x 10^{-4} M), DL-thiorphan (3 x 10^{-4} M), captopril (10^{-5} M), pepstatin A (10^{-5} M), PMSF (10^{-3} M), aprotinin (10^{-5} M), E-64 (10^{-5} M), cystatin (10^{-6} M), leupeptin (10^{-4} M), chymostatin (10^{-4} M) or bestatin (10^{-5} M)) were added to the baths 30 min before big ET-1 (1-39).

ET-1, ET-2 and ET-3 (n=6-12) were equipotent in causing contractions of the gallbladder. Big ET-1(1-39) induced slowly developing contractions and was only 4 to 6 fold less potent than ET-1 (apparent pD_2: 6.9 and 7.6, respectively; n=7-11). Big ET-2 was as potent as ET-2 at producing contractions (pD_2: 6.7 and 7.4, respectively; n=6-7) while ET-3 was >35 fold more active than big ET-3 (apparent pD_2: <6.0 and 7.4, respectively; n=6-12). The contractions induced by 3 x 10^{-7} M big ET-1 (1-39) were inhibited by 6, 26 and 58 % by 0.1, 1 or 3 x 10^{-4} M phosphoramidon, respectively (n=3). Phosphoramidon also shifted the concentration-reponse curve of big ET-1(1-39) to the right. Conversely, DL-thiorphan (a metalloprotease inhibitor), captopril (an angiotensin-converting enzyme inhibitor) or inhibitors of aspartic, serine, thiol (cysteine) proteases did not affect the response to big ET-1(1-39).

Thus, the guinea-pig gallbladder is not only very sensitive to the contractile effect of the endothelins but also contains a very high activity of ECE that is selective for big ET-1 and big ET-2 over big ET-3; it is also phosphoramidon-sensitive. These characteristics are the same as the membrane-bound phosphoramidon-sensitive neutral metalloprotease that has been proposed as the physiologically relevant ECE (Opgenorth et al., 1992) and purified from rat lung (Takahashi et al., 1993) and porcine aortic endothelial cells (Ohnaka et al., 1993). The guinea-pig gallbladder may therefore be a rich source of ECE for purification and characterization.

We thank Parke-Davis Pharmaceutical Research for a grant. BB is a recipient of Research Fellowship Awards from the National Heart and Stroke Foundation of Canada and the FCAR du Québec.

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494P [^{125}I]PD151242 AS AN ENDOTHELIN ET_A-SELECTIVE RADIOLIGAND IN THE HUMAN, RAT AND PORCINE HEART

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We have characterised the new ET_A selective radioligand [^{125}I]-PD151242 ((N-[(hexahydro-1-azepinyl)carbonyl])L-Leu-(1-Me)D-Trp-D-Tyr; Davenport *et al.* 1994) using the previously described human, rat and porcine heart model systems which have been shown to express both the endothelin ET_A and ET_B receptor subtypes (Peter & Davenport 1994).

Association experiments were carried out using slide-mounted cryostat tissue sections with fixed concentrations of [^{125}I]-PD151242 (2000 Ci/mmol; Amersham) for increasing time periods (0 - 240 min). In dissociation experiments heart sections were incubated with [^{125}I]-PD151242 for 2h before adding an excess of HEPES buffer for increasing time periods (0 - 240 min and 24 h). For saturation experiments tissue sections were incubated with 8pM - 8nM [^{125}I]-PD151242 for 120 min and for competition experiments with 0.1 nM [^{125}I]-ET-1 (2000 Ci/mmol; Amersham) together with increasing concentrations of PD151242. Non-specific binding was defined by the use of 1μM unlabelled ET-1 or PD151242, respectively. Binding parameters were calculated using the non-linear iterative curve fitting programme LIGAND. Results are given as mean ± s.e.mean. All experiments were carried out at room temperature (23°C).

Binding of [^{125}I]-PD151242 to sections of human left ventricle was time dependent and reached equilibrium within 2 hours with observed association rate constant k_{obs} = 0.0172 min^{-1}. The dissociation rate constant (k_{-1}) was determined to be 1.44×10^{-3} min^{-1}, yielding an estimate for the association rate constant of 0.0235 $min^{-1} \times nM^{-1}$. Non-specific binding was less than 15%. Saturation experiments revealed dissociation constants in the nM range for the human left ventricle (K_D = 1.07±0.08 nM), rat heart (K_D = 0.64± 0.08 nM) and porcine left ventricle (K_D = 1.92±0.27 nM) (n=3). Analysis of the binding data by LIGAND indicated that a one-site model was preferred to a two- or three- site fit. The Hill coefficients for [^{125}I]-PD151242 were close to unity for these three species, suggesting the presence of a single population of binding sites. Competition binding studies with unlabelled PD151242 indicated the presence of two binding sites with a 10,000 fold selectivity for the higher affinity binding site. PD151242 binds with a 6-fold lower affinity for the ET_A-receptor (K_DET_A = 7.21±2.83 nM, n=5) compared to other ET_A-selective antagonists such as FR139317 and BQ123 (Molenaar *et al.* 1993, Peter & Davenport 1994).

The iodination of PD151242 does not result in a decrease in the affinity for the ligand to the receptor: The K_D obtained using the iodinated PD151242 compound is approximately 1 nM, while the K_DET_A from competition binding studies against [^{125}I]-ET-1 is 7.2 nM. Furthermore, [^{125}I]-PD151242 showed reversible binding making it a useful radioligand for the ET_A receptor.

We are grateful to the theatre staff at Papworth Hospital for permission to collect human tissue samples and we thank the Schering Research Foundation and SERC for their support.

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495P CHARACTERISATION OF VASOCONSTRICTOR ENDOTHELIN RECEPTORS IN HUMAN ISOLATED RENAL ARTERY AND VEIN

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Autoradiographical studies have shown that whilst the endothelin (ET) ET_B receptor is distributed throughout the human kidney the ET_A receptor localises to the renal vasculature (Karet et al., 1993). In rats renal vasoconstriction appears to occur through activation of either subtype (Bennett et al., 1993). We have used reverse transcriptase polymerase chain reaction (RT-PCR), autoradiographical and *in vitro* pharmacological methods to identify the endothelin receptors present in human main branch renal artery and vein and to determine which receptor subtypes mediate endothelin-induced vasoconstriction.

RT-PCR was carried out using nested oligonucleotide primers as described (Davenport et al., 1993). For autoradiographical visualisation of ET receptors 10μm cross-sections of artery and vein were incubated for 2 hours at 22°C with 0.2nM [^{125}I]ET-1, or the ET_A selective antagonist [^{125}I]PD151242 (Davenport et al., 1994) or the ET_B agonist [^{125}I]BQ3020 (Bennet et al., 1993). Non-specific binding was defined by inclusion of 1μM unlabelled peptides. Functional studies employed 2mm rings of endothelium-denuded human renal artery and vein mounted in organ baths containing Krebs-Henseleit solution (37°C). Cumulative dose response curves (DRC's) were constructed to ET-1, ET-3, sarafotoxin 6b (S6b) and the ET_B-selective agonist sarafotoxin 6c (S6c). Peptide responses were expressed as a % of the contraction elicited by 50mM KCl.

RT-PCR detected mRNA encoding both ET_A and ET_B receptors in media of artery and vein (n=3). Both [^{125}I]ET-1 and [^{125}I]PD151242 bound to sections of artery and vein but little was observed with [^{125}I]BQ3020 suggesting a majority of ET_A receptors . ET-1 (10^{-10}-3×10^{-7}M)) produced dose-dependent contractions of renal artery and vein with EC_{50} values of 3.7 (1.3-10.4)nM and 1.1 (0.5-2.5)nM respectively (geometric mean ± 95% C.I. n=7). S6b was 10 times less potent in each case. 5/6 artery preparations failed to respond to 300nM ET-3 or S6c indicating that vasoconstriction in the renal artery is via the ET_A receptor. Responses to ET-3 and S6c in the vein were more variable. Veins from 3 individuals did not respond to 300nM ET-3 but in 3 further preparations DRC's were obtained, two of which were biphasic. ET-3 was always less potent than ET-1. S6c contracted 3/4 vein preparations, EC_{50} = 0.2 (0.07-0.54)nM (mean ± 95% CI), but in each case the maximum response was 30-60% of that to ET-1. These results suggest that although constrictor ET_B receptors may occur, the ET_A response predominates.

We have demonstrated that renal artery and vein contain both ET_A and ET_B receptor mRNA but that vasoconstriction *in vitro* appears to be due largely to ET_A receptor activation. An ET_A antagonist may therefore be of therapeutic benefit in conditions of reduced renal blood flow such as renal ischaemia or cyclosporine-induced toxicity.

We are grateful to the Theatre staff at Addenbrooke's and Hinchingbrooke Hospitals for permission to collect renal samples.

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496P ENDOTOXIN INDUCES RENAL VASOCONSTRICTION IN THE ANAESTHETISED RAT: EFFECT OF THE NON-SELECTIVE ENDOTHELIN RECEPTOR ANTAGONIST, PD 145065

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Endothelin (ET)-induced vasoconstriction in the rat kidney is mediated by both ET_A and ET_B receptors (Wellings *et al.*, 1993). Because plasma endothelin levels are raised in sepsis (Sugiura *et al.*, 1989), and endotoxin induces renal vasoconstriction (Kikera *et al.*, 1986), we have investigated the role of ET-1 in endotoxin-induced renal vasoconstriction in the rat, using a non-selective ($ET_{A/B}$) endothelin receptor antagonist, PD 145065 (Cody *et al.*, 1993).

Male Wistar rats (280-320g) were anaesthetised with thiopentone sodium (120mg/kg, i.p.). The carotid and femoral arteries, and jugular vein were cannulated for mean arterial pressure (MAP), blood sampling and infusion of drugs. Glomerular filtration rate (GFR) and renal blood flow (RBF) were measured according to the method of McNally *et al* (1990); [3H]-p-aminohippuric acid (PAH) (220KBq) and [14C]-inulin (22 KBq) were given as a bolus followed by infusion for 3h (440 KBq/h PAH and 44 KBq/h inulin). To show that PD 145065 blocked the renal effects of ET-1, vehicle or PD 145065 (1.5 mg, bolus) was given and then infused for 3h (0.05mg/min/rat). A 2h infusion of ET-1 (4 pmol/min/rat) or vehicle (0.1 % BSA in 0.9% saline) was started 1h after the beginning of the isotope and drug infusions. GFR and RBF were calculated from inulin and PAH clearance. To study the renal effects of endotoxin, conscious rats (n=5-7 per group) were injected with LPS (1 mg/kg, i.p.) or vehicle (0.9% saline). Rats were anaesthetised 5h later and cannulated for measurement of MAP, GFR and RBF 9h after LPS injection. The effect on renal function of infusing either PD 145065 (0.05 mg/min/rat) or vehicle was determined.

Table 1. Effect of PD 145065 on ET- responses (*$p<0.05$ v 1, $^{+}p<0.05$ v 2, ANOVA, n=4-6)

	GFR (ml/min)	RBF (ml/min)	MAP (mmHg)
(1)vehicle/vehicle	4.1 ± 0.5	16.0 ± 1.7	116 ± 8.7
(2)vehicle/ET-1	1.2 ± 0.1*	5.7 ± 0.5*	166 ± 1.3*
(3)PD 145065/vehicle	3.3 ± 0.4	15.5 ± 1.6	103 ± 9.5
(4)PD 145065/ET-1	$3.8 \pm 0.5^{+}$	$14.0 \pm 1.9^{+}$	$117 \pm 3.4^{+}$

LPS treatment did not affect MAP, but at 9h caused significant ($p < 0.05$) decreases in GFR (1.8 ± 0.2 *v* 3.2 ± 0.4 ml/min, controls) and RBF (8.5 ± 0.7 *v* 14.1 ± 2 ml/min, controls). PD 145065 at a dose that blocks the effects of ET-1, did not reverse the LPS induced falls in GFR (1.9 ± 0.3 ml/min) or RBF (8.7 ± 1.3 ml/min), suggesting a role for other mediators in the renal dysfunction caused by LPS.

We thank Parke-Davis Pharmaceutical Research for funding this work and the Chemistry Dept. for providing PD 145065.

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497P IL-1 RECEPTOR ANTAGONIST INHIBITS THE ENDOTOXIN-INDUCED RISE IN PLASMA IL-6 IN THE RAT

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Interleukin-1 (IL-1) is a potent mediator of acute inflammatory reactions, including fever. It is, however, difficult to detect significant amounts of IL-1 in the circulation of febrile animals and it has been suggested that IL-1 may act by inducing a secondary circulating pyrogen, such as IL-6. Injection of neutralising anti-IL-1 antibodies attenuates the febrile response induced by endotoxin, and inhibits the release of IL-6 into the circulation (LeMay et al, 1990). More recently, Smith & Kluger (1992) found that injection of the competitive IL-1 receptor antagonist (IL-1ra, Synergen, Colorado, USA) inhibited the rise in temperature in the rat, but did not significantly inhibit plasma IL-6. Since this suggested that IL-6 may not be an important physiological pyrogen, we have investigated further the effects of IL-1ra on fever and IL-6 release, and have found that kinetic considerations may have been overlooked.

Male, Sprague Dawley rats, weighing 250-300g, were injected intraperitoneally (ip) with 100μg/kg endotoxin, followed by two injections of IL-1ra (4mg/rat,ip), 1h and 2h after endotoxin treatment. Core temperatures were monitored for 4h using remote telemetry via pre-implanted abdominal transmitters after which the rats were killed and plasma collected for bioassay of IL-6. Another group of animals received a single dose of IL-1ra (4mg/rat,ip) 1h after endotoxin and were killed at 2h. Control animals were injected with 0.9% saline and/or IL-1ra at the appropriate time.

A single injection of endotoxin (100μg/kg) resulted in a rise in body temperature which peaked at 4h and was sustained for up to 24h. Core temperature increased by 1.5 ± 0.2°C (mean$\pm$sem) above basal values 4h after endotoxin administration. Treatment with IL-1ra significantly attenuated the rise in temperature, by 90% at 2h ($P<0.01$) and by 50% at 4h ($P<0.05$). Plasma IL-6 concentrations measured 4h after endotoxin were significantly elevated compared to controls (endotoxin 9878 ± 3372 U/ml; saline 13 ± 3 U/ml, $P<0.01$). Treatment with IL-1ra reduced, but did not significantly inhibit, the IL-6 concentrations in plasma (7759 ± 2281 U/ml). However, in animals that were killed 2h after endotoxin injection (1h following IL-1ra treatment), plasma IL-6 was significantly reduced (endotoxin 36070 ± 12850 U/ml; endotoxin+IL-ra 6955 ± 2000 U/ml $P<0.01$).

These results support the involvement of IL-1 in the development of fever and IL-6 release induced by endotoxin. They also indicate, that since pyrogenic activity of IL-6 is delayed, the short plasma half-life of IL-1ra (<20min) may be responsible for the previous failure to observe a significant reduction in IL-6 at the time of maximum fever.

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498P PULMONARY VASCULAR AND BRONCHIAL ACTIONS OF ENDOTHELINS AND SARAFOTOXIN 6c IN THE RAT ISOLATED PERFUSED LUNG

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Endothelins (ETs) are known to exert powerful vasoconstrictor effects (Horgan et al., 1991), they also stimulate bronchial smooth muscle (Matsuse et al., 1990). The present study has compared the actions of endothelin-1 (ET-1), endothelin-2 (ET-2), endothelin-3 (ET-3) and sarafotoxin 6c (SX6C) in the ventilated perfused rat lung. Lungs were removed from anaesthetized male Wistar rats (300-350 g) and set up to continuously record pulmonary perfusion pressure (PPP), pulmonary inflation pressure (PIP) and lung weight (LW) (Lal et al., 1994). After 30 min stabilisation bolus doses of agents (10-100 μl) were injected into the pulmonary artery. Only one agent was studied in each preparation.

ET-1 was most potent in raising PPP (ED_{50} 130.2$\pm$17pmol, n=10) compared with ET-2 (239.7$\pm$19 pmol, n=4) and ET-3 (722$\pm$77 pmol, n=4). However, SX6C a selective ET_B receptor agonist (ED_{50} 165$\pm$19 pmol,n=6) was of similar potency to ET-1. Doses of ETs required to produce a 50% increase in LW were ET-1, 206.6$\pm$25 pmol,n=10; ET-2, 427.6$\pm$12 pmol,n=4 and ET-3, 820$\pm$58 pmol,n=4 while SX6C (50-800 pmol) caused little change in LW. Bronchoconstrictor activities of these substances as reflected by changes in PIP again revealed differences in the potencies of SX6C and ETs: SX6C was extremely potent (ED_{50} 80.6$\pm$6.4 pmol, n=6) and it also produced a higher maximum response than any ET. The ED_{50} values for ET-1, ET-2 and ET-3 on PIP were 183$\pm$22 pmol, 377$\pm$66 pmol and 268$\pm$43 pmol respectively (n=4-10). In addition the slope of the PIP response to SX6c was significantly steeper than that of any of the ETs ($p<0.001$).

The potency profiles of the ETs on PPP indicate that ET_A receptors are involved in causing pulmonary vasoconstriction. Changes in LW also appear to be mediated by ET_A receptors, indeed such changes are probably a consequence of intense venoconstriction producing hydrostatic oedema (Rodman et al., 1992). However the finding that SX6c produced pulmonary vasoconstriction without marked increases in LW suggests that ET_B receptors are also involved and that they have a different distribution from ET_A receptors within the pulmonary circulation and are probably located on the arterial vessels. The bronchoconstrictor actions of ETs and SX6C appears to be mediated via (heterogenous ?) ET_B receptors. The reasons for the differences in the bronchial and vascular actions of SX6C and ETs are currently under investigation.

H.Lal is an Indian government Scholar.

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499P ADENOSINE TRIPHOSPHATE, ADENOSINE AND 2-CHLOROADENOSINE INHIBIT ENDOTHELIN-1 RELEASE FROM CULTURED ENDOTHELIAL CELLS

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The potent vasoconstrictor peptide endothelin-1 (ET-1) has been implicated in a number of cardiovascular diseases. Hence, agents inhibiting ET-1 release may prove useful in the treatment of ET-1 dependent pathologies. The regulation of ET-1 release is unusual in that it appears to be exerted primarily at the level of gene transcription, with newly synthesised peptide being secreted rapidly. High rates of shear stress and nitric oxide have been shown to inhibit ET-1 release from cultured cells (Kuchan & Frangos, 1993), but other factors suppressing ET-1 synthesis have yet to be identified. Because adenosine and ATP have vasodilator effects on the majority of blood vessels, with the action being exerted partially by the endothelium in some vessels (Gordon & Martin, 1983), we have investigated the effect of ATP, adenosine and analogues of these compounds on ET-1 release from cultured endothelial cells.

Endothelial cells from bovine aorta up to passage 2 (BAEC) and the human endothelial cell line EA.hy 926 were cultured in 6 x 35 mm well plates and used when confluent (Corder *et al.*, 1993). Cells were treated for 24 h in serum free medium at the concentrations of each agent indicated. Results are means ± sem from 2 - 5 experiments with triplicate determinations in each. The conditioned medium was harvested and stored frozen until RIA for ET-1 or big ET-1 (Corder *et al.*, 1993). ET-1 mRNA levels were estimated by the RNAse protection assay technique (McMahon *et al.*, 1993).

Control ET-1 release from BAEC and EA.hy 926 cells was 1614 ± 96 and 436 ± 34 fmol/well/24h respectively. In both cell types ATP, adenosine or 2-chloroadenosine (2-CADO) caused a concentration-dependent inhibition of ET-1 release, but the selective P_2 purinoceptor agonists α,β-methylene adenosine 5′-diphosphate and adenosine 5′-O-(2-thiodiphosphate) had no significant effect. 100μM ATP, adenosine or 2-CADO inhibited ET-1 release from BAEC by 48 ± 9, 19 ± 6 and 55 ± 5 % compared to control. The corresponding reductions for EA.hy 926 cells were 42 ± 6, 21 ± 4, and 67 ± 3 %. The adenosine analogues, N^6-cyclohexyladenosine and N^6-cyclopentyladenosine, were found to have no greater effect than adenosine, and 5′-(N-ethylcarboxamido)-adenosine was inactive at concentrations up to 1 mM. Measurement of big ET-1 levels in the conditioned medium from EA.hy 926 showed the effect of adenosine on ET-1 to occur in parallel with suppression of big ET-1 release, indicating an effect on proendothelin-1 synthesis rather than an action on processing. To test this further, mRNA levels for ET-1 were measured in EA.hy 926 cells after 1, 2 and 4 h treatment with 300 μM 2-CADO. ET-1 mRNA levels were reduced by between 75% and 50% compared to the corresponding control values. These results indicate that the inhibitory actions of ATP, adenosine and 2-CADO are mediated via adenosine receptors rather than P_2 subtype purinoceptors, however the involvement of nucleotide receptors in the response to ATP cannot yet be excluded. Adenosine appears to act by reducing the transcriptional rate for ET-1, or by increasing the rate of ET-1 mRNA degradation, but additional actions may be exerted elsewhere in the ET-1 biosynthetic pathway.

This work was supported by the Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co.
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500P PIVOTAL ROLE OF PHOSPHATE CHAIN LENGTH AS A DETERMINANT OF VASOCONSTRICTOR OR VASODILATOR ACTIVITY OF ADENINE NUCLEOTIDES IN THE RAT MESENTERIC ARTERIAL BED

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Purine compounds elicit vasoconstriction via P_{2x}-purinoceptors on the smooth muscle and vasodilatation via endothelial P_{2y}-purinoceptors in the rat mesenteric arterial bed (Ralevic & Burnstock, 1988). Vascular reactivity of the adenine dinucleotides, which are known to have diverse pharmacological effects (Hoyle, 1990), was examined to determine which class of purinoceptors they activate and the relationship between structure and activity. The following dinucleotides were tested: β-nicotinamide adenine dinucleotide (NAD); β-nicotinamide adenine dinucleotide phosphate (NADP); flavin adenine dinucleotide (FAD); adenylyl adenosine (AP1A); P^1, P^2-diadenosine pyrophosphate (AP2A); P^1,P^3-diadenosine triphosphate (AP3A); P^1,P^4-diadenosine tetraphosphate (AP4A); P^1,P^5-diadenosine pentaphosphate (AP5A); P^1,P^6-diadenosine hexaphosphate (AP6A).

Arterial beds were perfused at 5 ml/min with gassed (95% O_2/5% CO_2) Krebs' solution at 37°C. Responses were measured as changes in perfusion pressure (mmHg). The adenine nucleotides had the following order of vasoconstrictor potency at basal tone: α,β-methylene ATP (α,β-meATP) > AP5A > AP6A > AP4A > 2-methylthio ATP (2meSATP) > ATP > ADP. NAD, NADP, FAD, AP1A, AP2A and AP3A had no effect on basal tone. pD_{30} values (-log mol evoking increase in perfusion pressure of 30 mmHg) for AP4A, AP5A and AP6A were 7.9 ± 0.16 (n = 6), 8.5 ± 0.15 (n = 8) and 8.2 ± 0.09 (n = 7) respectively. Only one constrictor was tested per preparation to avoid auto- and cross-desensitization of responses to α,β-meATP, AP4A, AP5A, AP6A and ATP; a single dose of AP5A (15 nmol) reduced responses to consecutive doses of these nucleotides by approximately 60%. Vasoconstrictor responses to noradrenaline were unaffected. Responses to AP5A were significantly inhibited by the selective P_{2x}-purinoceptor antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS, 1-10 μM); estimated pA_2 value for antagonism of AP5A by PPADS was 5.72. Responses to AP4A, AP5A, AP6A and α,β-meATP were virtually abolished by PPADS at 30 μM. A significant component of the vasoconstrictor response to ATP was resistant to blockade by PPADS at 30 μM. In raised tone preparations (induced by 10-30 μM methoxamine, n = 21) the following order of vasodilator potency was observed: 2meSATP > ADP > ATP > AP3A > AP2A > AP1A > NADP > FAD >> NAD. pD_{20} values (-log mol evoking decrease in perfusion pressure of 20 mmHg) for AP1A, AP2A and AP3A were 7.1 ± 0.23 (n = 8), 7.5 ± 0.21 (n = 10) and 8.5 ± 0.18 (n = 10) respectively. Removal of the endothelium with sodium deoxycholate abolished these responses. AP4A, AP5A, AP6A and α,β-meATP had no vasodilator effects.

In conclusion, the adenine dinucleotides AP4A, AP5A and AP6A elicit vasoconstriction in the rat mesenteric arterial bed via P_{2x}-purinoceptors. AP1A, AP2A, AP3A, FAD and NADP elicit vasodilatation via receptors present on endothelial cells which are likely to be of the P_{2y}-purinoceptor subtype. NAD had no activity. It is suggested that there is a crucial relationship between the structure and activity of these purine compounds that hinges on the phosphate chain length and its possible charge – molecules with three or more phosphates are vasoconstrictors, while those with three or less phosphates are vasodilators.

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501P CONTRACTILE ACTIONS OF PYRIMIDINE AND PURINE NUCLEOTIDES IN THE RAT PULMONARY VASCULAR BED

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The naturally occurring pyrimidine nucleoside triphosphates have been shown to have vascular effects via P_{2u}-purinoceptors (Abbracchio et al., 1993). The presence of specific P_{2x}-purinoceptors, which mediate the contractile action of ATP, has been demonstrated on several vessels, including the rat pulmonary vascular bed (Burnstock, 1993). This study investigates the contractile effects of the pyrimidine nucleotides uridine, cytidine and thymidine 5'-triphosphate (UTP, CTP and TTP) and compares them with the actions of the purine nucleotides adenosine, guanosine and inosine 5'-triphosphate (ATP, GTP and ITP) on the rat pulmonary vascular bed. It also aims to identify the receptors mediating the actions of these agents.
Lungs of male Wistar rats (300-350 g) were isolated and perfused via cannulation of the pulmonary artery at a constant flow rate of 8 ml/min. Vascular responses were detected as changes in perfusion pressure (mm Hg) following bolus injection of 100 µl of the drug tested. In preparations at basal tone (14.7±2.1 mm Hg; n=24), UTP (0.01-10 µmol) produced a dose-dependent vasoconstriction up to 8.0±0.9 (n=6) mm Hg. ATP was active at the same doses, inducing a maximal vasoconstriction of 9.4±0.8 (n=6) mm Hg. CTP and TTP (0.1-10 µmol) were less potent vasoconstrictors. At the highest dose tested, they induced a vasoconstriction of 5.7±0.5 (n=6) and 4.9±0.8 (n=6) mm Hg, respectively. The vascular responses to GTP were superimposable on those to CTP and TTP. ITP produced weak responses only at high doses. EC_{50} values were: 0.8, 0.6, 2.6, 2.1, 1.6 and 4.5 µM for ATP, UTP, CTP, GTP, TTP and ITP, respectively. In order to characterize the purinoceptors involved in the effects observed, the action of the most potent pyrimidine and purine nucleotides were compared in different experimental conditions. In the presence of the P_2-purinoceptor antagonist, suramin (100 µM) vascular responses to 1 and 10 µmol UTP were not significantly different from control responses. Further, the selective P_{2x}-purinoceptor antagonist, PPADS (pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid; 30 µM) did not affect the vasoconstriction to UTP and only weakly reduced vascular responses to 1 µmol ATP from 4.0±0.1 (n=4) to 3.1±0.3 (n=4) mm Hg. Twenty min perfusion of the preparations with 10 µM α,β-methylene ATP, which selectively activates and desensitises P_{2x}-purinoceptors, partially reduced the vasoconstriction to 1 µmol ATP, without affecting UTP vasoconstriction.

This study indicates that pyrimidine and purine nucleotides produce vasoconstriction of rat pulmonary vascular bed. The order of potency of these agents was: UTP = ATP > TTP ≥ GTP ≥ CTP > ITP. These results also suggest the presence of a suramin-resistant P_{2u}-purinoceptor, which can be equally activated by UTP and ATP on the rat pulmonary vasculature.

This work was supported by the British Heart Foundation.

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502P P_2 PURINOCEPTOR ANTAGONIST EFFECTS OF PYRIDOXAL-5-PHOSPHATE IN THE RAT VAGUS NERVE AND GUINEA-PIG AORTA

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Recent evidence suggests that pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) exerts antagonist effects at P_2 purinoceptors (Lambrecht *et al.*, 1992). In the present study we have examined the antagonist properties of a synthesis precursor of PPADS, pyridoxal-5-phosphate, at P_2 purinoceptors. The effects of this agent were determined on depolarisation responses of the rat vagus nerve induced by α,β-methylene adenosine 5'-triphosphate (α,βmeATP; Trezise *et al.*, 1993) and relaxant responses of the guinea-pig aorta induced by 2-methylS-ATP (Dainty *et al.*, 1992), effects thought to be mediated via P_{2X}- and P_{2Y}- purinoceptors, respectively.

Depolarisation responses of the rat vagus nerve were measured using a 'grease-gap' technique, as previously described (Trezise *et al.*, 1993). Concentration-effect curves to α,β-meATP were constructed by addition of sequentially increasing doses of agonist to the perfusate for periods of 2-3min at 45min intervals. Standard isometric force recording methods were used to measure the relaxant effects of 2-methylS-ATP in guinea-pig aortic rings. A single cumulative concentration-effect curve to 2-methylS-ATP was constructed in each preparation, pre-contracted with a submaximal concentration of phenylephrine (1μM), in the presence of indomethacin (3μM) and 8-*p*-sulfophenyltheophylline (30μM). In the rat vagus nerve, α,β-meATP (0.3-300μM) produced concentration-related depolarisation responses with a geometric mean EC_{50} value of 14.9μM [10.8-20.4], n=13. Pyridoxal-5-phosphate (10, 30 and 100μM) caused concentration-dependent rightward displacements of the concentration-effect curves to α,β-meATP, which were incomplete, with analysis of the lower portions yielding mean concentration ratios (CRs) of 3.3 [1.0-11.0], 5.6 [1.8-17.3] and 20.7 [9.5-45.4], respectively (n=4-6). In contrast, depolarisation responses to 5-HT were not attenuated by pyridoxal-5-phosphate (100μM; CR 1.3 [1.0-1.8], n=4). In the guinea-pig aorta, 2-methylS-ATP produced relaxation with a mean EC_{50} value of 30.2nM [11.4-87.7], n=7. In the presence of pyridoxal-5-phosphate (10, 30 and 100μM) the concentration-effect curves to 2-methylS-ATP were displaced to the right in a parallel manner, analysis yielding CRs of 2.7 [0.8-8.5], 8.5 [3.0-24.0] and 24.4 [9.7-61.6], respectively (n=6-7) and resulting pA_2 and slope values of 5.39±0.30 and 0.97±0.29, respectively (mean ± s.e.mean). Relaxant responses to the adenosine (P_1) agonist, 5'-N-ethyl carboxamideadenosine, were not markedly attenuated by pyridoxal-5-phosphate (100μM; CR 1.9 [0.9-4.0]; n=5).

These data indicate that pyridoxal-5-phosphate exhibits specific antagonist actions at P_2 purinoceptors of both the P_{2X} and P_{2Y} subtypes. This compound is, thus, the smallest molecule so far described that has blocking effects at P_2 purinoceptors. Although in some systems the usefulness of this agent may be limited by its profound effects on certain intracellular biochemical processes (see Stryer, 1975 for overview), pyridoxal-5-phosphate may prove to be a useful starting point in the synthesis of novel, low molecular weight P_2 purinoceptor antagonists.

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503P EVIDENCE THAT ATP AND β,γ-METHYLENE ATP PRODUCE RELAXATION OF GUINEA-PIG TRACHEALIS VIA AN ACTION INVOLVING P_1 PURINOCEPTORS

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ATP and analogues have been shown to produce relaxation of isolated guinea-pig trachealis (Welford & Anderson, 1988), possibly via P_2 purinoceptors (Fedan et al., 1993). Our aim was to investigate further whether ATP and analogues cause relaxation via an action at P_2 purinoceptors and, if so, characterise that receptor.

Segments of trachea were mounted for isometric recording in organ baths containing a physiological salt solution plus nitrobenzylthioinosine (NBTI, 300 nM) and indomethacin (2.8 μM) maintained at 37°C. Cumulative log concentration effect curves were constructed to agonists against carbachol (300 nM)-induced spasm and responses expressed as a percentage of the relaxation produced by aminophylline (1 mM). Tissues were incubated with antagonists (P_2 purinoceptor : suramin - 90 min; P_1 purinoceptor : 8-sulphophenyltheophylline [8-SPT] - 60 min) or vehicle before concentration effect curves to agonists were repeated.

The potency order for relaxation induced by ATP and analogues was β,γ-meATP = ATP > α,β-meATP >> UTP = 2meS-ATP. Suramin (100 μM) had no effect on responses to ATP or β,γ-meATP (ATP: $_pD_2$= 4.45 ± 0.11 [control], 4.26 ± 0.08 [+ suramin], n = 6; β,γ-meATP: $_pD_2$=4.7 ± 0.04 [control], 4.6 ± 0.07 [+ suramin], n = 6, P > 0.05). Both ATP and β,γ-meATP were significantly antagonised by 8-SPT (140 μM), although full log concentration-effect curves to both agonists could not be constructed in the presence of 8-SPT. Adenosine and its synthetic analogue 5'-N-ethylcarboxamidoadenosine (NECA) also produced relaxation of guinea-pig trachealis and their potency was not affected by suramin (100 μM) (adenosine: $_pD_2$= 4.75 ± 0.14 [control], 4.51 ± 0.11 [+ suramin], n = 4; NECA: $_pD_2$= 6.06 ± 0.05 [control], 6.13 ± 0.06 [+ suramin], n = 4, P > 0.05). Adenosine and NECA were each antagonised by 8-SPT (140 μM) (adenosine: log CR = 1.37 ± 0.14, n = 4; NECA: log CR = 1.69 ± 0.03, n = 6).

These data suggests that ATP and β,γ–meATP produce relaxation of isolated guinea pig trachealis by activating the same receptors as adenosine and NECA (presumably P_1 purinoceptors) and provides no evidence for the existence of P_2 purinoceptors in this tissue.

We appreciate the support of a DENI studentship (A.S.K.) and from Glaxo (G.I.A.P.).

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504P THE EFFECT OF CHRONIC PRETREATMENT ON THE ANTIARRHYTHMIC AND HAEMODYNAMIC EFFECTS OF THE A_1-ADENOSINE AGONIST, R-PIA, IN RAT ISOLATED HEARTS

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The antifibrillatory effect of acute administration of the A_1-adenosine agonist, R-PIA, has been demonstrated previously *in vivo* (Wainwright & Parratt, 1993). Sustained exposure of A_1 receptors to an A_1 agonist causes receptor down-regulation in rat adipocytes (Green, 1987). Should this occur in the heart it could render A_1 agonists unsuitable as long-term antiarrhythmic therapy. The aim of the present study was to compare the antiarrhythmic and haemodynamic effects of R-PIA in rat isolated hearts with those in hearts from rats which had been treated chronically with R-PIA. Male rats were anaesthetized with sodium pentobarbitone, the hearts excised and perfused in the Langendorff mode (constant flow; 8-9ml min^{-1}) and the left main coronary artery occluded to induce regional ischaemia. For the acute studies five experimental groups were performed: (i) Controls and R-PIA at concentrations of (ii) 10^{-9}M, (iii) $5x10^{-9}$M, (iv) 10^{-8}M and (v) $5x10^{-8}$M (n=10 for all groups) added to the perfusate 10 min before occlusion. In the chronic studies rats were pretreated with either saline injection (0.25ml i.p. b.d.for 7 days; controls, n=10) or R-PIA ($50\mu gkg^{-1}$ i.p. b.d. for 7 days; n=10). At the end of treatment the hearts were prepared as above and perfused with R-PIA 10^{-8}M. Arrhythmia count (VEB's), incidences of ventricular tachycardia (VT) and fibrillation(VF), and heart rate (HR) and perfusion pressure (PP) were recorded for 30 minutes

Table 1: $^{*}P<0.05$ vs acute control (Fisher Irwin). $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs acute control (ANOVA)

GROUP	VEB's	%VT	%VF	HR (Pre-drug)	%ΔHR(postdrug)	PP (Pre-occlusion)	$\%\Delta PP_{max}$
Acute Perfusion							
Control	431±127	100	60	344±5	-1±3	54±6	110±20
R-PIA 10^{-9}M	736±273	80	10*	331±12	-17±2	38±4	47±20#
R-PIA $5x10^{-9}$M	453±164	70	20*	325±11	-26±3#	44±3	49±12#
R-PIA 10^{-8}M	256±125	70	10*	331±14	-36±4##	51±4	54±13#
R-PIA $5x10^{-8}$M	264±92	50*	0*	281±13	-42±4##	52±7	40±8#
Chronic Treatment + perfusion with R-PIA (10^{-8})							
Saline i.p.	364±191	70	20*	317±11	-32±3##	40±3	52±16#
R-PIA $50\mu gkg^{-1}$	230±85	80	0*	338±11	-15±6	46±5	59±13#

These results demonstrate that R-PIA exerted a dose related effect on HR, but the antifibrillatory effect and attenuation of the PP response to occlusion occurred independently of dose in the range tested. Following chronic treatment with R-PIA there was an attenuation of the HR response to R-PIA, suggesting A_1 receptor down-regulation. The suppression of VF and the ischaemia-induced rise in PP was unaffected by chronic administration. These results suggest that the antifibrillatory action of R-PIA is mediated by a mechanism unrelated to heart rate.

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505P CARDIOVASCULAR EFFECTS OF GR79236, APNEA AND CGS21680 IN THE ANAESTHETISED RAT

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Recently, adenosine agonists have been suggested to mediate a hypotensive effect in pithed rats by activation of a xanthine-insensitive receptor - the putative adenosine A_3 receptor (Fozard and Carruthers, 1993). In the present study, the cardiovascular effects of selective A_1 and A_{2a} agonists and effects of the antagonists 8-p-sulphophenyltheophylline (8SPT) and CGS15943A (9-fluoro-2-(2-furyl)-5,6-dihydro[1,2,4]triazolo{1,5-c}-quinazin-5-imine) on these effects were examined in the anaesthetised rat.

Female normotensive AH/A rats (200-250g) were anaesthetised and prepared for recording of blood pressure (BP) and heart rate (HR). Agonists were injected intravenously (iv) and dosing was cumulative; antagonists or vehicle were injected iv 5 min prior to starting the agonist dose-response curve (DRC). Only one agonist DRC was generated per animal.

Table 1 **Cardiovascular effects of adenosine agonists and their blockade by 8SPT and CGS15943A (mean + 95% confidence interval)**

	Control		8SPT-treated[c]		Dose-ratio			CGS15943A-treated[d]		Dose-ratio		
	BP ED_{40}[a] (nmol/kg)	HR ED_{40}[a] (nmol/kg)	BP ED_{40}[a] (nmol/kg)	HR ED_{40}[a] (nmol/kg)	BP[b]	HR[b]	n	BP ED_{40}[a] (nmol/kg)	HR ED_{40}[a] (nmol/kg)	BP[b]	HR[b]	n
GR79236[e]	25 (9-72)	23 (14-37)	307 (104-896)	496 (328-750)	12	21	5	302 (131-697)	466 (315-689)	12	20	4
APNEA[f]	25 (14-43)	164 (44-615)	27 (19-38)	774 (497-1205)	1	5	5	27 (6-120)	>880	1	>7	4
CGS21680[g]	7 (4-12)	>1000	90 (27-298)	>1000	13	-	5	947 (498-1799)	>3000	135	-	4

(a) dose to lower BP and HR by 40% (b) BP or HR ED_{40} (control)/BP or HR after antagonist (c) 40mg/kg or (d) 3mg/kg given 5min prior to agonist DRC (e) N-[(1S,trans)-2-hydroxycyclopentyl]adenosine (f) N^6-2-(4-aminophenyl)ethyladenosine (g) 2-({2-[4-(2-carboxyethyl)phenyl]ethyl}amino)-N-ethylcarboxamidoadenosine

The A_3 agonist APNEA, and the A_1 receptor-selective agonist GR79236 produced a dose-dependent fall in both BP and HR. The A_{2a} agonist CGS21680 produced a fall in BP, but had no effect on HR. Following either 8SPT (40mg/kg) or CGS15943A (3mg/kg) the hypotension and bradycardia to A_1 agonists was reduced. However, the hypotension produced by APNEA appeared to be independent of HR, whereas that to GR79236 appeared to be dependent on HR. CGS15943A was a more effective antagonist of the hypotensive effects of the A_{2a} agonist CGS21680 than was 8SPT (see Table 1).

In conclusion, GR79236 is a selective adenosine A_1 receptor agonist which only produces hypotension at doses which exert bradycardic activity. In addition, the hypotensive effects of APNEA which are resistant to blockade by 8SPT are also resistant to blockade by CGS15943A.

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506P THE NOVEL MONOSODIUM SALT OF 7-NITROINDAZOLE INHIBITS BRAIN NITRIC OXIDE SYNTHASE AND ELEVATES STRIATAL DOPAMINE EFFLUX *IN VIVO*

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The non-specific nitric oxide synthase (NOS) inhibitor L-N^G-nitro arginine methyl ester potentiates N-methyl-D-aspartate-mediated release of dopamine (DA) in rat striatum *in vivo* (Rose *et al.*, 1993) suggesting a role for nitric oxide (NO). Recently, Moore *et al.* (1993) reported an inhibitory effect of 7-nitroindazole (7-NI) on cerebral NOS activity with no effect on mean arterial blood pressure, thus suggesting a specific inhibitory effect on the brain enzyme. However, 7-NI cannot be used to study neurotransmitter release *in vivo* due to its insolubility in artificial CSF (aCSF) at concentrations above 100μM. We now report the use of a more soluble salt of 7-NI in studying the effect of inhibition of NOS in rat cerebellar homogenates and on basal DA release *in vivo* using microdialysis.

7-NI was dissolved in ethanol/diethyl ether and reacted with equimolar NaOH. The resulting salt was evaporated, purified by solvent extraction and examined for purity by TLC, NMR and MS. NOS activity was measured in rat cerebellar homogenates by the conversion of [^{3}H] L-arginine to [^{3}H] citrulline (Moore *et al.*, 1993). Striatal DA efflux was measured by *in vivo* microdialysis in chloral hydrate (500mg/kg ip) anaesthetised rats as previously described (Rose *et al.*, 1993). Probes were continuously perfused with aCSF at a rate of 2μl/min. Following a 1 h period, 20μl fractions were collected into 25μl 0.25M acetic acid. Following determination of basal DA efflux over a 1 h period, 7-NI monosodium salt (7-NINA; 1mM, n=6) or vehicle (aCSF, n=5) was incorporated into the aCSF for the remainder of the perfusion period (90 min). Dialysate samples were assayed for DA by HPLC.

Both 7-NINA and 7-NI caused a dose-related inhibition of NOS activity (IC_{50} = 0.4 μM and 0.7μM respectively). Perfusion with 7-NINA increased striatal DA efflux to 128% above basal (198±60 compared to 87±12 fmol/20μl) immediately on incorporation of the drug (Figure 1). Maximum increase in efflux of dopamine was achieved 60 min following the start of perfusion with 7-NINA (210% above basal; 271±90 compared to 87±12 fmol/20μl) and remained raised for the remainder of the perfusion period (Figure 1). DA efflux was not altered in control animals.

7-NINA provides a useful tool for the study of NO activity in brain. Salt formation appears not to alter the ability of 7-NI to inhibit NOS. The present data support the concept that inhibition of NO formation enhances DA efflux in the rat striatum. This is contrary to studies empoying rat striatal slices (Hanbauer *et al.*, 1990), but consistent with reports of the role of NO in the rat striatum *in vivo* (Rose *et al.*, 1993).

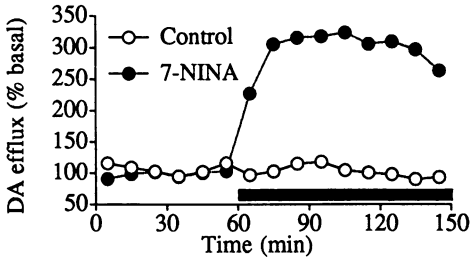


Figure 1 The effect of 7-NINA (1mM; bar) on DA efflux in rat striatum *in vivo*. 7-NINA increased DA efflux compared to control ($p<0.05$, repeated measures ANOVA).

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507P THE EFFECTS OF A PHOTOSENSITIVE NITRIC OXIDE DONOR ON DOPAMINE EFFLUX MEASURED BY FAST CYCLIC VOLTAMMETRY IN THE RAT STRIATUM

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Recent evidence suggests a role for nitric oxide (NO) in the control of dopamine release in the striatum (Zhu & Luo, 1992). Roussins Black Salt (RBS) has been shown to be a photosensitive donor of NO (Flitney *et al.*, 1992; Matthews *et al.*, 1994) and the effects of supplying NO to the brain slice in this manner on dopamine efflux were investigated.

Brain slices (350μm) containing the striatum were prepared from male AHA Sprague-Dawley rats (150-200g). Slices were placed in a perspex bath superfused with oxygenated artificial CSF at 32°C (flow rate = 1.2ml min^{-1}). Dopamine levels were monitored using fast cyclic voltammetry as described by Palij and colleagues (1990). RBS (30μM) was added to the perfusion medium for 30min and washed off for 30min before illumination protocols were commenced. The experiment was performed under darkened conditions throughout.

Illumination of the brain slice in the absence of RBS had no effect on dopamine efflux. RBS in the absence of illumination caused a slight reduction (20±11%, n=14, mean ± sem) in electrically stimulated dopamine efflux with no discernible effects on the baseline dopamine levels over a 30min period. After 30min washout of the RBS, a rapid (reaching plateau in approx. 1-2min) light intensity-related increase in dopamine baseline levels (159Lux 26±5nM; 1370Lux 80±18nM; 4040Lux 141±27nM; n=14) was seen with white light illumination. This effect was reversed upon cessation of the illumination. Analysis of the voltammetric current traces revealed similar signals evoked by RBS illumination, exogenous and endogenous dopamine. Associated with the increase in baseline was a marked reduction in the electrically stimulated levels of dopamine post illumination (62±5%, n=14). This effect was repeated after 30min in the presence of oxyheamoglobin and sulpiride.

Group	*Increase in baseline dopamine (nM)*			*% decrease in stimulated dopamine*	
	159Lux	*1370Lux*	*4040Lux*		
control (n=6)	32±9	82±15	219±60	49±8	
10μM oxyhaemoglobin (n=4)	4±3*	40±4*	108±15	50±13	
1μM sulpiride (n=4)	21±7	61±13	224±96	69±4	*p<0.05 ANOVA

The increase in baseline dopamine levels was attenuated by the presence of oxyhaemoglobin, but not affected by sulpiride. The decrease in stimulated dopamine efflux was not affected by the presence of either drug. These data suggest that RBS can be retained in brain tissue to release NO when required in response to illumination. It would appear that NO delivered in this way causes an increase in dopamine efflux with concommitant attenuation of dopamine released by electrical stimulation; the latter effects do not involve dopamine D_2 autoreceptors.

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508P EFFECT OF THE PUTATIVE DOPAMINE D_3 RECEPTOR AGONIST, (+)-PD 128907, ON PREPULSE INHIBITION OF ACOUSTIC STARTLE RESPONDING IN THE RAT

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Whilst previous studies have clearly demonstrated that dopamine receptor agonists attenuate prepulse inhibition of acoustic startle responding, the nature of the receptor subtype involved remains unclear. Recent radioligand binding studies suggest that (+)-PD 128907 has both high affinity (Ki = 1.2nM) and good selectivity (>1500 fold) for human D_3 receptors (DeMattos et al., 1993). The present study has compared the effects of (+)-PD 128907 with those of the mixed D_2/D_3 receptor agonists, apomorphine, quinpirole and 7-OH-DPAT, on prepulse inhibition.

Startle responses were recorded in male Wistar rats (300 - 500 g) using 6 SR-LAB (San Diego Instruments) stabilimeter chambers. On experimental days, rats were housed in an adjacent holding room and exposed to white noise (65 dB) for at least 1 hour prior to testing. All drugs were administered subcutaneously and rats were placed in individual startle chambers 10 min later. Following a 5 min acclimation period to background noise (65 dB), startle responses were recorded to 15 repetitions of 4 different stimuli: i) prepulse (80 dB tone, 5 msec duration), ii) prepulse + pulse (140 dB tone, 40 msec duration, beginning 100 msec after onset of the prepulse), iii) pulse alone and iv) a period when no stimulus was presented. Stimuli were presented in a random order with a mean interstimulus interval of 15 sec. Mean startle amplitude ± s.e. mean for each stimulus type was calculated for each subject (n = 10 - 15 rats per group) and prepulse inhibition calculated as the % reduction in startle responding ie 100 x [pulse - (prepulse+pulse)]/pulse (analysed by ANOVA followed by Dunnett's t test, * = $P<0.05$ compared to vehicle pretreated rats).

Rats pretreated with vehicle showed a mean reduction of 59% in amplitude of the startle response when a 140dB stimulus (pulse) was preceded by an 80 dB prepulse (pulse = 720 ± 42; prepulse + pulse = 295 ± 30*). Furthermore, prepulse inhibition was dose-dependently antagonised by apomorphine (% inhibition: veh = 56.2 ± 4.2; 0.1 mg/kg = 31 ± 5.7*; 0.3 mg/kg = 19 ± 7.4*; 1 mg/kg = 4.6 ± 6.6*), quinpirole (veh = 56 ± 6; 0.01 mg/kg = 39 ± 5; 0.03 mg/kg = 13 ± 6*; 0.1 mg/kg = 11 ± 7*) and 7-OH-DPAT (veh = 65 ± 3; 0.01 mg/kg = 43 ± 6; 0.03 mg/kg = 26 ± 6*; 0.1 mg/kg = 8 ± 13*) at doses which did not significantly alter startle responses to the pulse alone. Significant reversal of prepulse inhibition was also observed following treatment with (+)-PD 128907, although the maximal effect was considerably less than that of mixed D_2/D_3 receptor agonists (% inhibition: veh = 62 ± 4; 0.03 mg/kg = 46 ± 7; 0.1 mg/kg = 32 ± 7*; 0.3 mg/kg = 36 ± 7*) suggesting that the compound may be a partial agonist at the D_3 receptor. Pretreament with higher doses did not result in further inhibition of this response (1 mg/kg = 31 ± 7%; 3 mg/kg = 31 ± 5%) but significantly attenuated startle responses to the pulse alone (veh = 844 ± 77; 1 mg/kg = 430 ± 45*; 3 mg/kg = 473 ± 51*).

In conclusion, the present results suggest that activation of D_3 receptors may contribute towards the ability of dopamine receptor agonists to attenuate prepulse inhibition in the rat. However, in vitro studies confirming that (+)-PD 128907 has functional D_3 receptor selectivity and the use of selective D_3 receptor antagonists are required to clarify the role of the receptor in this response.

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509P TWO CALCIUM CHANNEL ANTAGONISTS, NITRENDIPINE AND FELODIPINE, EXHIBIT OPPOSITE ACTIONS AGAINST THE *IN VIVO* EFFECTS OF THE POTASSIUM CHANNEL BLOCKER 4-AMINOPYRIDINE

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We have shown previously that dihydropyridine calcium channel antagonists, such as nitrendipine, can reduce the ethanol withdrawal syndrome (Little et al., 1986). Felodipine, another dihydropyridine calcium channel antagonist, however did not show this effect (Watson and Little, 1994a). In the present study we examined the effect of felodipine and nitrendipine on the convulsions induced by bicuculline and the potassium channel blocker 4-aminopyridine (4-AP). The doses of felodipine and nitrendipine used (10 and 50 mg/kg respectively) caused the same displacement of dihydropyridine [3H]-isradipine from CNS tissue and reduced the convulsive behaviour induced by central administration of the calcium channel agonist Bay K 8644 (Watson et al., 1992)

Groups of 10 - 15 male TO mice (25-30 g) were used. A 30 min i.p. pretreatment with felodipine, nitrendipine, or 0.5% Tween vehicle was given. The threshold dose for elicitation of a clonic convulsion was determined by intravenous infusion of the convulsant drug via a tail-vein. Incidence of convulsions was measured following i.p. injection of bicuculline 2.7 mg/kg or 4-AP 8 mg/kg. The latencies from injection of bicuculline 3.5 mg/kg or 4-AP 8 mg/kg until a full convulsion were also timed. Statistical analysis was by Student's unpaired t-test, or Fisher's exact probability test.

Table 1: Tween (Tw), felodipine 10 mg/kg (fel), nitrendipine 50 mg/kg (nit) *P < 0.05 compared with controls.

Convulsant	Convulsion Threshold (mg/kg)			Convulsion incidence			Latencies to convulsion (s)		
	Tw	Fel	Nit	Tw	Fel	Nit	Tw	Fel	Nit
Bicuculline	0.42±0.03	0.54±0.04 *	0.55±0.02*	1/8	0/8	0/8	63±8	114±17*	102±13*
4-AP	85 ± 6.1	84 ± 6.8	104 ± 5.2*	4/10	9/10 *	3/10	650±60	800±75	1250±75*

Both felodipine and nitrendipine raised the convulsive thresholds for infusion of bicuculline, and increased the latencies to convulsions after i.p. injection of bicuculline. Nitrendipine also increased the convulsion threshold and latencies to convulsions produced by 4-AP. Felodipine, however, did not alter the convulsion threshold for infusion of 4-AP and was proconvulsant to the i.p. injection of 4-AP, increasing the number of animals convulsing. These changes were unlikely to be due to any effect on blood-pressure, as mice treated with hexamethonium 8 mg/kg did not show increased thresholds to bicuculline (control 0.50 ± 0.04 mg/kg; hexamethonium 0.52 ± 0.04 mg/kg)

We have shown previously that the convulsion thresholds for 4-AP were raised during ethanol withdrawal (Watson and Little, 1994b). It is possible therefore that the lack of effect of felodipine during ethanol withdrawal may be related to its proconvulsant action on convulsions induced by 4-AP.

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510P THE EFFECT OF PROPOFOL ON EPILEPTIFORM ACTIVITY INDUCED BY ZERO MAGNESIUM IN THE CORTICAL SLICE *IN VITRO*

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Propofol is a relatively fast acting, intravenous general anaesthetic with rapid recovery. Associated side effects are few but occasionally convulsions on induction or recovery have been noted. Laboratory data illustrate an anticonvulsant activity although Dolin *et al* (1992) and Bansinath *et al* (1992) have demonstrated proconvulsant profiles against strychnine and kainate/quisqualate seizures *in vivo*, respectively. The *in vitro* brain slice offers a more simplified approach and allows a post-dosing effect to be investigated. Hence the aim of this study was to examine the effect of propofol on spontaneous epileptiform activity induced by zero magnesium in cortical wedges *in vitro*.

Cortical wedges taken from Sprague Dawley rat brains were placed in a grease gap chamber so that cortex and subcortex were perfused separately from one another with aCSF, 2ml/min; subcortical perfusate contained 1mM $MgCl_2$. Epileptiform activity consisted of an initial burst with afterpotentials (APs) superimposed on the decay phase recorded with Ag/AgCl electrode inserted into the back of each chamber. Bursts and AP number were counted for 5 minutes every 15 minutes throughout the experiment and expressed as burst/min and AP/burst. The control period consisted of four counts (0, 15, 30 and 45 mins). Propofol (5.6, 16.8, or 56μM in aCSF) was added at 59 mins and counts taken at 60 and 75 minutes. At 89 mins propofol was removed and a washout period of 3 hours then followed with regular 5 minute counts. Intralipid (Kabi Pharmaceuticals), 1 in 1000 dilution was included as vehicle and time matched control experiments were conducted following the same protocol. Statistical analysis was by MANOVA with repeated measures, *post-hoc* t tests with Bonferroni correction (* $p<0.005$, ** $p<0.0025$).

Overall control bursting frequency was 4.3 ± 0.25/min and AP frequency was 6.3 ± 0.45 per burst (n=42). All groups were $n>8$. Control bursting frequency increased over the experimental period (4.2 ± 0.58 to 5.5 ± 0.59) and AP/burst decreased (4.6 ± 0.62 to 1.7 ± 0.34). Intralipid had no effect on bursting frequency or AP number compared to control. Propofol dose dependently suppressed spontaneous activity and at the highest dose (56μM) reduced bursting frequency by 88% and AP number by 100%. Afterpotentials did not return for 1 hour after removal of drug but were back at control levels by the end of the experiment (255 mins). In contrast bursting frequency recovered more quickly and increased above the control level in the latter stage of washout (Fig 1).

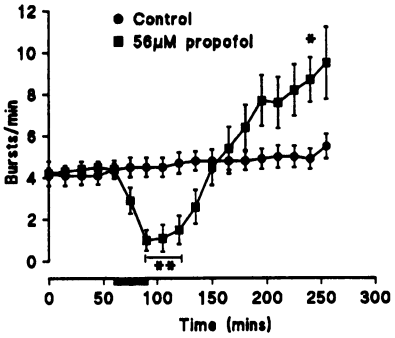


Figure 1: Effect of 56μM propofol on Bursts/min (Bar shows propofol dose time)

These data would suggest that although propofol produces a prolonged anticonvulsant effect, a rebound in bursting activity is observed which may represent excitatory activity.

The financial support of Zeneca is gratefully acknowledged.

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511P THE EFFECT OF HALOTHANE ON [^{3}H]NORADRENALINE RELEASE FROM SH-SY5Y HUMAN NEUROBLASTOMA CELLS

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Whilst the precise cellular mechanism of action of volatile anaesthetic agents (eg halothane) remains to be determined a reduction in neurotransmission may have role to play in anaesthesia (Griffiths & Norman, 1993). We have examined the effects of halothane on K^+ and carbachol evoked [^{3}H]noradrenaline (NA) release from a human neuroblastoma cell line, SH-SY5Y. All experiments were performed in whole cells at 37^{o}C in Krebs/HEPES buffer pH 7.4. using a perfusion system. Buffers were equilibrated with halothane delivered by a precalibrated Fluotec 3 vaporiser at settings 1-5%. Aqueous halothane concentrations exposed to the cells were determined by gas chromatography (Rutledge *et al.*, 1963). Cells were incubated with 50nM NA for 60mins, washed with 3x1ml aliquots of buffer then loaded into the perfusion chamber and perfused at 0.5ml/min (Atcheson *et al.*, 1994). To assess the effects of halothane on K^+ evoked NA release we examined the effect of this agent on the S2/S1 stimulus ratio for a pair of 100mM K^+ pulses separated by 15mins. For carbachol (1mM) evoked release a single challenge (S1) was used. Cells were exposed to halothane for 15min prior to stimulus, either S1 (carbachol) or S2 (K^+).

Both K^+ and carbachol produced a monophasic release profile with greater release ocurring with K^+. K^+ evoked release was inhibited dose-dependently by halothane, Table 1.

Table 1. Effect of halothane on NA release.

[Halothane] μM (Atm %)	% Inhibition of K^+ release	Carbachol release
235.7 (0.84)	5.2±0.6	6.6±1.6
353.2 (1.26)	15.5±0.7	
523.2 (1.87)	27.6±2.6*	+10.3±3.2
645.8 (2.31)	27.6±3.5*	
893.9 (3.19)	46.6±6.1*	13.6±2.3

Data are mean±s.e. mean n=5-9. Atm% is calculated assuming at equilibrium 1% Atm gives an aqueous concentration of 280μM (Franks & Lieb, 1993). *P<0.05 compared with air alone. + represents stimulation.

These data indicate that only K^+ evoked release is inhibited by halothane. Furthermore, as carbachol but not K^+ evoked release in these cells is extracellular Ca^{2+} independent (Atcheson *et al.*, 1994) our data suggest that the target site for this agent may be a Ca^{2+} channel.

R. Atcheson is a British Journal of Anaesthesia Research Fellow.

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512P KAINATE-EVOKED PURINE RELEASE FROM RAT HIPPOCAMPUS

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The neurotoxicity produced by systemic administration of kainate can be prevented by R-PIA. This protection is prevented by the A1 receptor antagonist CPX, at doses which do not themselves enhance kainate toxicity (MacGregor and Stone, 1994). This implies that endogenous adenosine exerts little protective action against kainate toxicity and we now report a direct examination of adenosine release in the hippocampus.

Male Wistar rats (270-310g), anaesthetised with urethane, had concentric dialysis probes stereotaxically inserted into the hippocampus (5.6mm P, 5.0mm L, 8.0mm V, relative to the bregma). ACSF was perfused at 2µl/min. Samples were collected every 20 min and analysed for purine content by h.p.l.c.. Basal levels became stable 2hr after probe insertion.

The administration of 1mM kainate into the perfusing solution induced a release of adenosine (Ogilvy & Stone, 1993) which reached 255.98±28.98% (mean±s.e.mean) of basal during a first stimulus (S1) and 165.43±21.68% during a second stimulus (S2). The S2:S1 ratio was reduced significantly to 0.04±0.03 by CNQX (4.5µM, n=4), and to 0.31±0.05 by ascorbic acid (10mM, n=4). Dizocilpine (MK-801) (0.1mM, n=4), and R-PIA (10µM, n=4) present during S2 did not significantly reduce the S2:S1 ratio. Potassium-evoked release was unaffected by CNQX (4.5µM). Unpaired t-test was employed and significance level was $P<0.05$.

The adenosine release by kainate implies an extracellular concentration in the hippocampus of about 2µM, which would probably be insufficient to cause significant protection.

This work was supported by the Scottish Home and Health Department.

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513P DIFFERENCES BETWEEN THE HIGH AFFINITY [^{3}H] α,β-METHYLENE ATP BINDING SITES IN RAT VAS DEFERENS AND RAT BRAIN

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[^{3}H]α,β-methyleneATP ([^{3}H]αβmeATP) binds to both high and low affinity sites in rat vas deferens and it has been suggested that the high affinity sites are P_{2X} purinoceptors (Bo et al., 1992; Michel & Humphrey, 1993). High affinity binding sites for [^{3}H]αβmeATP have also been identified in rat brain (Michel & Humphrey, 1993). In this study we have compared the [^{3}H]αβmeATP binding sites in rat vas deferens and brain membranes, using 1nM [^{3}H]αβmeATP as described previously (Michel & Humphrey, 1993), except that a 50mM Tris 4mM $CaCl_2$ 1mM EDTA assay buffer (pH 7.4 at 4°C) was used. All data are the mean±s.e.mean (n=3-5).

Several compounds appeared to differentiate between the [^{3}H]αβmeATP binding sites in vas deferens and brain (see table). Of particular interest were the data obtained with αβmeADP. In vas deferens both αβmeATP and αβmeADP identified a single population of sites at which αβmeADP displayed low affinity (see table). In brain, however, both αβmeATP and αβmeADP identified two populations of sites. Furthermore both compounds identified the same ($P>0.05$; Students t test) proportion of sites (αβmeADP=53.4±5.7%; αβmeATP=63.4±3.0 %) with high affinity and it appeared that the high affinity αβmeATP sites (pIC_{50}=8.32±0.05) also possessed high affinity for αβmeADP (pIC_{50}=7.08±0.14). Thus, in the presence of 100nM αβmeATP, to occlude the high affinity αβmeATP binding sites, αβmeADP possessed low affinity (pIC_{50}=4.76±0.14), while in the presence of 1µM αβmeADP, to occlude sites with high affinity for αβmeADP, αβmeATP possessed low affinity (pIC_{50}=6.55±0.19).

Compound	αβmeATP	αβmeADP	APPPP *	Dextran Sulphate
Vas deferens	8.3±0.2 (0.85±0.03)	3.2±0.3 (0.81±0.11)	8.4±0.1 (0.85±0.03)	6.2±0.1 (0.40±0.04)
Brain	7.7±0.1 (0.50±0.01)	5.9±0.1 (0.40±0.03)	6.8±0.1 (0.64±0.03)	4.0±0.1 (0.41±0.01)

Date are pIC_{50}'s with Hill slopes in parenthesis. * APPPP is adenosine tetraphosphate.

These data therefore suggest the presence of more than one high affinity αβmeATP binding site. One site, in the vas deferens, may correspond to functional P_{2X} purinoceptors since αβmeADP is about 1000 times less potent than αβmeATP as an agonist in this tissue (Fedan et al., 1982). However, the high affinity αβmeATP binding sites that we have identified in the brain are different since they have high affinity for αβmeADP. In view of the low potency of αβmeADP as a P_{2X} purinoceptor agonist in functional studies (Fedan et al., 1982) it is possible that the high affinity αβmeATP sites in brain are not P_{2X} purinoceptors. However, it is interesting to note that, in rat colon longitudinal muscle, both αβ meADP and αβmeATP stimulate an atypical P_2-purinoceptor mediating relaxation (Bailey & Hourani, 1992) and further study will be required to determine if the high affinity [^{3}H]αβmeATP binding sites in brain are similar.

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514P ADENOSINE RECEPTOR ANTAGONISTS ATTENUATE THE $[K^+]_e$ INCREASE DURING HYPOXIA IN THE RAT HIPPOCAMPUS *IN VITRO*

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In the CA1 region of the rat hippocampal slice, hypoxia induces a suppression of synaptically evoked potentials, pyramidal cell hyperpolarisation and an increase in extracellular K^+ concentration ($[K^+]_e$, Hansen *et al.*, 1982). We investigated the action of two adenosine receptor antagonists on all of these effects since the first of these appears to be mediated by adenosine (Fowler, 1989).

Transverse hippocampal slices (350 μm) were prepared from anaesthetised male Sprague-Dawley rats (70-110 g, pentobarbitone 440 mg/kg). Slices were submerged in a chamber (volume 0.5 ml) and superfused (2-3 ml/min at 30 °C) with an aqueous solution containing (mM) NaCl 125, KCl 2, KH_2PO_4 1, $MgSO_4$ 1, glucose 5, $NaHCO_3$ 25, $CaCl_2$ 2.5 gassed with either 5% CO_2 in O_2 (control) or 5% CO_2 in N_2 (hypoxia). $[K^+]_e$ was measured by ion-selective, valinomycin-based, double-barreled electrodes (tip 5-8 μm). Recording electrodes were placed in the CA1 stratum pyramidale. Near-maximal field potentials were evoked every 30s by a stimulating electrode placed in the CA3 stratum radiatum, the evoked potential being recorded via the reference barrel of the ion-selective electrode. Intracellular recordings were made in separate experiments using 3M KCl-filled microelectrodes (55-110 MΩ). Each slice was subjected to a single period of hypoxia (5 mins) with or without drug pretreatment (30 mins). We routinely confirmed that the antagonists had reduced the hyperpolarisation or suppression of evoked potentials induced by adenosine (10-30 μM). Data is presented as median (range, number). A significant effect was attributed to $P<0.05$ in the Mann-Whitney U-test.

Hypoxia increased $[K^+]_e$ by 1.3 mM (1.1-1.4, n=5) from 3mM. Coincident with the initial rise in $[K^+]_e$ was a reduction and eventual abolition of the field potential. Treatment with theophylline (100μM) or 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 100 nM) prevented hypoxia-induced transmission failure and significantly reduced the increase in $[K^+]_e$ caused by this insult: 0.6 mM (0.4 to 0.8, n=4) and 0.6 mM (0.5 to 0.9, n=5), respectively. Under control conditions pyramidal cells had membrane potentials of -69 mV (-51 to -73, n=13) and hypoxia induced a hyperpolarisation of 6 mV (3 to 10, n=13). Treatment with theophylline or DPCPX induced a slight depolarisation and epileptiform activity of cells but the pooled membrane potentials before hypoxia were not significantly different from controls : -65 mV (-58 to -74, n=8) and -67 mV (-59 to -77, n=5), respectively. Neither treatment significantly reduced the hypoxia-induced hyperpolarisation: 10 mV (8 to 14, n=8) in theophylline and 10 mV (4 to12, n=5) in DPCPX; actually, they may have increased it.

In conclusion, we can confirm that A_1 receptor activation is involved in the hypoxia-induced depression of excitatory transmission (Fowler, 1989) but seems unlikely to contribute to the hypoxia-induced hyperpolarisation (cf. Spuler & Grafe, 1989). In addition, it seems that these receptors contribute significantly to the hypoxia-induced increase in $[K^+]_e$ in the CA1 pyramidal cell layer.

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515P THE SUSCEPTIBILITY OF AGONIST-EVOKED RESPONSES TO ANTAGONISM BY (±) CP96345 AND RP67580 DIFFERS FOR HUMAN AND RAT CLONED NK_1 RECEPTORS

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It has been proposed that for cloned NK1-receptors that different agonists may interact with different sites on the receptor, leading to differences in the effects of NK1-receptor antagonists (Huang *et al.*, 1994; Pradier *et al.*, 1993). We compared the effects of (±) CP96345 and RP67580 on $Sar^9Met^{11}(O_2)$ SP and septide evoked changes in total IP levels using rat (rNK1) and human (hNK1) cloned NK1 receptors.

hNK1 and rNK1 receptors were stably expressed in CHO cells and rat mutant (L116V, S290I) (Fong *et al* 1992) NK1 receptors were transiently expressed in COS-7 cells. Changes in IP levels (in the presence of 10mM LiCl) were measured following incubation (3d) with inositol myo-[2-^{3}H(N)]. For the hNK1, rNK1 and rNK1(L116V, S290I) receptors both septide and $Sar^9Met^{11}(O_2)$ SP evoked 3-5 fold changes in IP levels and the EC_{50} values (95% confidence limits; nM) for $Sar^9Met^{11}(O_2)$ SP were 1.3 (1.0-1.6), 0.7 (0.5-1.1) and 0.5 (0.4-0.6) and for septide were 6.3 (4.0-10.0), 7.9 (3.7-17.0) and 6.0 (3.3-10.9) respectively ($n > 4$). The pA_2 values for (±) CP96345 and RP67580 are shown in Table 1.

Table 1. pA2 values (95% confidence limits) determined from Schild plots (+ indicates slope significantly different from 1); * indicates apparent pA_2 values obtained using single antagonist concentrations (1μM; n = 4 determinations).

	hNK1		rNK1		rNK1 (L116V,S290I)	
Agonist	(±) CP96345	RP67580	(±) CP96456	RP67580	(±) CP96345	RP67580
Septide	9.1 (9.0-9.2)	7.0 (6.9-7.1)*	7.7 (7.5-7.8)*	9.1 (9.0-9.2)+	9.5 (9.3-9.7)*	Not tested
$Sar^9Met^{11}(O_2)$ SP	9.0 (8.9-9.2)	6.9 (6.7-7.1)*	7.1 (7.0-7.2)*	8.1 (8.0-8.4)	8.8 (8.7-9.0)*	Not tested

Comparison of pA_2 values showed that for the hNK1 receptor, there was no difference in the antagonistic effects of (±) CP96,345 and RP67580 on changes in IP levels evoked by either agonist, although in some experiments a decrease in the maximum response to septide was observed. For the wild type and mutant rat receptors, IP changes evoked by septide were more susceptible to inhibition by the NK1-receptor antagonists. It is possible that for the rNK1 receptor different agonists interact with different epitopes on the receptor, whereas for the hNK1 receptor the binding epitopes for septide and $Sar^9Met^{11}(O_2)$ SP and the antagonists may share more similarity.

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516P ANTI-EMETIC ACTIVITY OF NEUROKININ NK_1 RECEPTOR ANTAGONISTS IS MEDIATED CENTRALLY IN THE FERRET

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It has been shown previously in the ferret that a neurokinin NK_1 receptor antagonist (NK_1 antagonist) administered peripherally exerts an anti-emetic action against a wide range of emetogens (Bountra *et al.*, 1993). In the present study, the effects of central administration of NK_1 antagonists (a peptide, GR82334, and non-peptides, racemic CP-99,994 and its less active enantiomer, CP-100,263) on emesis induced by cisplatin were investigated in the conscious ferret. In addition, substance P (SP) was administered centrally in non-cisplatin treated ferrets.

A guide-cannula was implanted in the hindbrain of adult, male ferrets (body weights 1.2-1.8kg) under general anaesthesia (Higgins *et al.*, 1989), and a minimum of 48h was allowed before experimental use. Emesis was induced by intraperitoneal cisplatin (200mg m^{-2} body surface area). The NK_1 antagonists or saline control were administered (dose volume, 2µl) after the first emetic response into the hind-brain, in the region of the nucleus tractus solitarius, via a needle passed through the implanted guide-cannula. The number of retches was recorded over a 4h period after central administration, and the results are shown in Table 1. Two non-cisplatin-treated ferrets received SP centrally (10µg in 2µl) on 7 occasions at 30min intervals.

Table 1

Treatment	Dose	n	Total retches (mean ±S.E.M.)	Inhibition (%)
Saline control	2µl	5	121±16.8	-
(±)CP-99,994	1µg	6	77±13.3†	36
	10µg	6	27±7.7†	78
CP-100,263	10µg	6	65±9.8†	46
GR82334	1µg	5	58±17.6†	53
	10µg	5	28±15.3†	77

† significant difference from control, $p<0.05$, unpaired t test

Racemic CP-99,994 produced a dose-related inhibition of cisplatin-induced emesis, and the 10µg dose of CP-100,263 showed similar anti-emetic activity to the 1µg dose of the racemate. Peripheral administration of GR82334 (5.4mg kg^{-1} i.v., n=4) showed no anti-emetic activity in cisplatin-treated ferrets, whereas central administration of the peptide produced inhibition. Repeated central administration of SP consistently induced an immediate, short-lived emesis (latency, <1min; duration, <5min; range 8-32 retches).

Thus, the central administration of NK_1 antagonists inhibits cisplatin-induced emesis in the ferret. Furthermore, SP induces emesis after central administration, and this effect can be observed repeatedly. The anti-emetic activity of GR82334 administered centrally, but not peripherally, indicates that the anti-emetic site of action of NK_1 antagonists is in the brain. These results support a central role for SP in the emetic reflex pathway of the ferret.

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517P OPIOID-MEDIATED STIMULATION OF [^{35}S]GTPγS BINDING IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS

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Guanine nucleotide exchange at heterotrimeric G-proteins is an early response in the signal transduction cascade following occupation of receptors of the 7-transmembrane domain superfamily by agonists. This can be measured by studying the stimulation of binding of [^{35}S]GTPγS to G-proteins by various agonists and in particular is readily measured for receptors coupled to adenylate cyclase, e.g. muscarinic m2 and m4 receptors (Lazareno & Birdsall, 1993). Opioid µ-receptors couple to various effector systems, including adenylate cyclase, *via* activation of Gi and Go proteins (Laugwitz *et al.*, 1993). It was of interest therefore to determine if µ-opioid agonists could stimulate [^{35}S]GTPγS binding and to characterise this response in SH-SY5Y human neuroblastoma cells, which endogenously express µ-opioid receptors (Kazmi & Mishra, 1987).

SH-SY5Y cells (passage number 75-85, expressing 262±19 fmols/mg protein µ-opioid receptors, measured using [^{3}H]DAMGO; [^{3}H][D-Ala^2,MePhe^4,Glyol^5]enkephalin) were grown in Modified Eagles Medium supplemented with 10% newborn and 2% foetal calf serum. Cells were harvested and membranes (50,000 x g) were prepared in Hepes buffer (10mM, pH 7.4) containing 100mM NaCl and 10mM $MgCl_2$. The binding of [^{35}S]GTPγS (80pM) was studied using 150-200µg membrane protein in the same buffer for 60 min at 30°C in the presence of 3µM GDP and varying concentrations of agonists. All values represent means ± sem.

The binding of [^{35}S]GTPγS to membranes from SH-SY5Y cells was stimulated by the µ-agonists fentanyl, DAMGO and morphine affording EC_{50} values of 15.2±3.0nM (n=8), 6.4±1.0nM (n=3) and 26.7±6.3nM (n=4) respectively. The dose-effect curves for these agonists were considerably to the left of their binding curves to low agonist affinity states of the µ-receptor (Carroll *et al.*, 1988). The dose-response curve for fentanyl was shifted to the right in the presence of the opioid antagonist naloxone affording a Ke for naloxone of 2.7±0.1nM (n=3) consistent with an action at µ-opioid receptors. Treatment of cells with pertussis toxin (100ng) for 24 h led to a complete loss of the agonist stimulation of [^{35}S]GTPγS binding, indicating the involvement of Gi and/or Go proteins. Indeed a 32.1±5.6% (n=3) reduction in basal [^{35}S]GTPγS binding was observed, indicating an endogenous stimulation of guanine nucleotide exchange even in the absence of added agonist. The partial agonists buprenorphine (EC_{50} 0.18±0.06nM, n=3) and pentazocine (EC_{50} 90.2±29.8nM, n=3) also stimulated [^{35}S]GTPγS binding but with a reduced efficacy affording 72.7±0.3% and 59.7±2.2% respectively of the maximal response to fentanyl. The results demonstrate that stimulation of [^{35}S]GTPγS binding can be used as a functional response for µ-opioid agonists and that it can distinguish agonists of differing efficacy.

We thank the Wellcome Trust for support.

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518P THE ARYLYAMINOPYRIDAZINE-GABA DERIVATIVE NCS 281-93 IS A POTENT GABA ANTAGONIST IN *ASCARIS SUUM*

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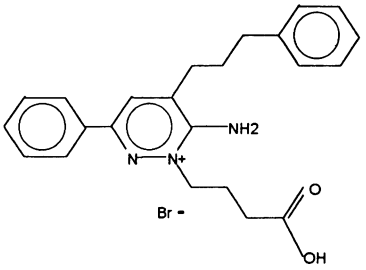


Figure1. NCS281-93 structure.

We have been examining the pharmacology of GABA antagonists in the nematode parasite *Ascaris suum* (Martin, 1993) with a view to finding a new approach for the development of anthelmintic compounds. A series of arylaminopyridazine-GABA derivatives were synthesised and their activity as GABA antagonists assessed electrophysiologically by measuring GABA-induced increases in chloride conductance in muscle cells (Duittoz and Martin, 1991). Within the series of compounds tested, NCS281-93 (Figure. 1), 2-(3-carboxy propyl)-3-amino-4-phenyl propyl: 6-phenyl pyridazinium bromide was found to be the most potent competitive GABA antagonist. The effect of different concentrations of NCS281-93 on GABA-chloride-conductance dose-response relationships was examined in muscle-flap preparations from seven adult female *Ascaris suum*. It was found that NCS281-93 produced a parallel shift to the right of the GABA dose-response curves. The data was analysed using a modified Schild equation (Duittoz and Martin, 1991) to describe the observations. The analysis allows for cooperativity of GABA and antagonist responses. The dissociation constant, K_B, was 4.7 $\pm$ 0.7μM (mean $\pm$ s.e.mean., N=11). The results demonstrate that potent antagonists of the nematode GABA receptor may be found in this series and offer a novel approach in the search for new anthelmintic agents.

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519P EFFECT OF INTERLEUKIN-4 ON INTERLEUKIN-1-INDUCED NEUTROPHIL MIGRATION AND ON ENDOTOXIN-INDUCED NITRIC OXIDE PRODUCTION IN MACROPHAGES AND NEUTROPHILS

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Recent therapeutic strategies in experimental septic shock include the inhibition of polymorphonuclear granulocyte (PMN) infiltration or function and the inhibition of nitric oxide (NO) synthase (NOS) induction or activity. Identification of anti-inflammatory cytokines which inhibit both processes may be of importance for the therapy of circulatory shock and chronic inflammation. We have studied the effect of interleukin-4 (IL-4) on (i) PMN migration and (ii) induction of NOS in macrophages and PMNs.

PMN migration was studied in an air pouch model in male Swiss albino mice (Perretti and Flower, 1993). Dorsal s.c. air pouches were prepared by the injection of 2.5 ml air on day 0 and day 3. Six days after the initial injection of air, animals received murine recombinant interleukin-1β (IL-1β, 5 ng) into the pouch. Separate groups of mice were pretreated with IL-4 (0.01-10 μg/mouse i.v.), 10 min prior to the injection of IL-1β. At 4h after the injection of IL-1β, mice were killed with CO_2 and lavage fluid was obtained from the pouches for the determination of PMN counts. The induction of NO formation caused by E coli endotoxin (lipopolysaccharide, LPS) was studied in mouse peritoneal macrophages *ex vivo* and *in vitro;* and in the J774.2 cell line *in vitro*. In other experiments, mice were treated with LPS (0.01-1 mg/kg) and peritoneal macrophages were obtained after 3h. Separate groups of mice were pretreated with IL-4 (0.01-10 μg/mouse i.v.) at 10 min prior to the injection of LPS. Macrophages were resuspended in DMEM and were placed in 96-well plates (400 000 cells/100 μl). Non-adherent cells were removed after 3h. Nitrite was measured by the Griess reaction at 24h in the culture medium (Szabo *et al.*, 1993). In the *in vitro* experiments, control macrophages were prepared as above or J774.2 macrophages were prepared as described (Szabo *et al.*, 1993) and stimulated with LPS (1 μg/ml) *in vitro* in the absence or presence of IL-4 (0.01-1 μg/ml). To study the effect of IL-4 on NO produced by PMNs, cells were obtained from air pouches stimulated with IL-1β in the presence or absence of IL-4. The cells were resuspended in DMEM, placed in 96-well plates (1 million cells/100 μl) and stimulated with LPS (1 μg/ml). Nitrite was measured at 24 h. Statistical analysis of the data (means ±SEM of 5-6 observations) was done by Student's t-test.

IL-1β induced a consistent and intense PMN migration into the air pouch at 4h (vehicle: 2.2±0.3; IL-1β: 9.4±0.7 million cells/pouch, $p<0.01$). This migration is not dependent on local production of NO, for it was not affected by local injection of the NOS inhibitor N^G-methyl-L-arginine (30-300 μg). Pretreatment with IL-4 (0.1-10 μg) caused an inhibition of PMN migration. For instance, at 1 μg of IL-4, migration was inhibited by 41±8% ($p<0.05$). LPS (0.01-0.3 mg/kg i.p.) induced a dose-dependent increase in nitrite production of peritoneal macrophages when measured *ex vivo* at 24h in the culture medium (basal:2.2±0.3 μM; LPS: 74.8±3.6 μM, $p<0.01$). This was inhibited by IL-4 (0.1-1 μg). For instance, at 1 μg IL-4, nitrite production was inhibited by 50±1% ($p<0.01$). IL-4 also significantly inhibited the accumulation of nitrite when NOS was induced by LPS *in vitro* in either peritoneal macrophages or in cultured J774.2 cells. Nitrite production by PMNs was far less than that by activated macrophages (17±4 μM at 24h after LPS) and was unaffected by IL-4 *ex vivo* or *in vitro*. Thus, (i) IL-4 inhibits the migration of PMNs in response to IL-1β; and (ii) inhibits NO production in LPS-activated macrophages, but not PMNs, *in vivo* and *in vitro* . These observations encourage the exploration of possible protective effects of IL-4 in various models of circulatory shock. (This work was supported by a grant of Glaxo Group Research Ltd. C.S. is supported by the Lloyds of London Tercentenary Foundation. Murine recombinant IL-4 was a generous gift from Schering-Plough Ltd., Ware, UK)

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520P INTERLEUKIN-5 INDUCES THE ADHESION OF EQUINE EOSINOPHILS TO FIBRONECTIN-COATED PLASTIC

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Eosinophils are the predominant leucocyte found in the dermis of horses with the allergic skin disease sweet itch after antigen challenge (Foster et al, 1992a). The mechanisms responsible for eosinophil recruitment into the skin are unknown. This study has examined the effect of mediators on the adhesion of equine eosinophils to plastic coated with the matrix protein fibronectin, since the adhesive properties of this cell type have not been established.

Microtitre plates (96-well) were sequentially coated with human fibronectin (10 μg ml^{-1}) and blocked with bovine serum albumin (20 μg ml^{-1}) (2% BSA), each for 1 h at 37°C. Human recombinant (hr) IL-5 (0.1 - 1000 ng ml^{-1}), hrC5a (10^{-10} - 10^{-7}M), PAF (10^{-10} - 10^{-6}M), LTB4 (10^{-10} - 10^{-6}M), fMLP (10^{-10} - 10^{-6}M) or vehicle (2% BSA) were added to test wells (n=6 for each concentration of agonist). After a 10 min incubation at 37°C eosinophils purified from normal horses (Foster et al, 1992b) were added to wells (1 x 10^5 cells/well) with or without pre-treatment with monoclonal antibody to the human adhesive receptor VLA4. Following incubation at 37°C for 25 min plates were washed and adherent cells lysed. The eosinophil peroxidase content of the cells was assayed by colorimetry at 490 nm after oxidation of o-phenylenediamine. The number of adherent cells was then calculated as a % of the total added to each well.

IL-5 (0.1 to 1000 ng ml^{-1}) induced a dose- and time-dependent increase in the adhesion of equine eosinophils to fibronectin-coated plastic ($p<0.01$; analysis of regression; n=5). The response was maximal at 25 min.

IL-5 (ng ml^{-1})	Vehicle	0.1	1	10	100	1000
% adhesion	0.2±0.05	0.2±0.05	0.7±0.2	1.7±0.4*	2.3±0.5***	2.6±0.8***

(Values are means ± s.e.mean, *$p<0.05$, ***$p<0.001$ compared to vehicle; Dunnett's test).
Antibody to human VLA4 (10μg ml^{-1}) inhibited IL-5 (100ng ml^{-1})-induced eosinophil adhesion by 44% (n=3). C5a (10^{-7}M) caused a small increase in the adhesion of eosinophils to fibronectin-coated plastic when compared with vehicle (1.1±0.1 vs 0.6±0.1%, $p<0.05$; Dunnett's test; n=3). PAF, LTB4 and fMLP did not induce eosinophil adhesion (n=5).

These findings show that IL-5 induces a small but significant increase in the adhesion of equine eosinophils to fibronectin-coated plastic and suggest that adherence is mediated in part via VLA4. This mechanism may play a role in the recruitment of equine eosinophils to the skin in sweet itch.

We are grateful to the Home of Rest for Horses for financial support.

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521P ROLE OF ENDOGENOUS CORTICOSTERONE IN PLATELET-ACTIVATING FACTOR-INDUCED NEUTROPHILIA IN THE MOUSE

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Intravenous (i.v.) treatment with platelet-activating factor (PAF), as with other polymorphonuclear cell (PMN) activators, induces a rapid and transient neutropenia followed by a sustained neutrophilia (e.g. Jagels & Hugli, 1992). Since glucocorticoids are known to have a potent neutrophilic action, this study investigated the potential role of endogenous corticosterone in PAF-induced neutrophilia in the mouse.

Normal or adrenalectomized (ADX) Swiss Albino mice (28-30g, Tuck, Essex, UK) were treated with a single bolus dose of PAF i.v, in 100 μl bovine serum albumin (BSA) 0.1% in sterile phosphate buffered solution (PBS). At different times mice were bled by cardiac puncture under halothane and then killed. Total white cell counts were determined using a Coulter counter and differential counts were obtained from blood smears stained with Turk's solution. In other cases, mice received corticotrophin (ACTH) by s.c injection and blood samples were taken 4h after injection. Plasma corticosterone (CCS) was assayed with a commercially available radioimmunoassay according to the manufacturer's instructions (ICN-Biomedical). Statistical comparisons on original values were made by unpaired Student's *t* test.

Circulating PMN levels in mice treated with BSA 0.1% alone were found to be 0.58 ± 0.04 x10^6 PMN, n=31, 0.73 ± 0.12 x10^6 PMN, n=16, and 1.49 ± 0.19 x10^6 PMN, n=57, $p<0.01$, at 2, 20 and 120 min, respectively (the PMN number for untreated mice was 0.64 ± 0.05 x10^6, n=23). PAF induced a dose-dependent (30-300 ng) neutropenia at 2 min, with an ED_{50} of 100 ng (50%, n=16, $p<0.01$), which had resolved at 20 min and was followed by a marked neutrophilia at 2h (+147%, n=6, $p<0.01$). PAF (100 ng) injection caused a significant rise in CCS levels maximal at 20 min: 235 ± 23 ngml^{-1}, n=7, $p<0.01$ *vs* naive mice (50 ± 6.5 ngml^{-1}, n=15) and $p<0.01$ *vs* BSA 0.1% treated animals (157 ± 14 ngml^{-1}, n=15). At 2 and 120 min PAF did not significantly alter CCS levels above that of vehicle injected mice (not shown). Adrenalectomy significantly reduced (50%) PAF-induced neutrophilia: 0.80 ± 0.14 x10^6 PMN n=6, and 1.41 ± 0.15 x10^6 PMN, (+76%), n=11; and 1.18 ± 0.23 x10^6 PMN, n=4, and 2.89 ± 0.58 x10^6 PMN (+145%), n=5, in ADX and naive mice for BSA 0.1% and PAF 100 ng, respectively. Subcutaneous administration of ACTH induced a dose-dependent (1-20 μg) neutrophilia with a maximal increase of +666% at 20 μg dose (n=11, $p<0.01$). This was mirrored by a rise in CCS levels (30 ± 9 ngml^{-1}, 501 ± 46 ngml^{-1} and 571 ± 49 ngml^{-1} for 1, 5 and 20 μg, respectively) which correlated with the effect on circulating PMN (correlation coefficient of 0.916).

In conclusion, this study investigated the effect of PAF (and ACTH) on circulating PMN in the mouse and suggests the involvement of endogenous CCS in this response. The molecular mechanism(s) related to these changes requires further elucidation.

The financial support of Ono Pharmaceutical Co. (Japan) is acknowledged. RJF is funded by an endowment from Lilly UK.

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522P INHIBITION OF INFLAMMATORY AND ALGESIC EFFECTS OF AN EXTRACT FROM *PORPHYROMONAS GINGIVALIS* BY THE BRADYKININ ANTAGONIST, ICATIBANT (Hoe 140)

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Porphyromonas gingivalis is a bacterium found in the bacterial flora of infected dental root canals and is significantly related to pain in periodontitis (Hashioka *et al.*, 1992). Several enzymes isolated from *P. gingivalis* have been reported to possess kininogenase actvity. One of these proteinases, lys-gingivain, a membrane-bound enzyme, apparently is the most potent kininogenase reported to date (Scott *et al.*, 1993). Since kinin release could be responsible for inflammatory symptoms and pain in periodontitis, we investigated whether the inflammatory and algesic effects of a sonic extract from *P. gingivalis* (PGSE; Scott *et al.*, 1993) could be inhibited by the potent bradykinin B_2 receptor antagonist, icatibant (DArg-[Hyp^3-Thi^5-DTic^7-Oic^8]-bradykinin; Lembeck *et al.*, 1991; former name Hoe 140).

In anaesthetized rats, the subplantar injection of PGSE (0.1 and 1.0 mg) caused a dose-dependent paw oedema. The net increase of paw volume (difference between PGSE-injected and control paw) 60 min after the injection was 23±5% and 77±12% (n=4–5), respectively. The oedema induced by 0.1 mg of PGSE remained unaffected (27±6%) by s.c. pretreatment with icatibant (300 nmol kg^{-1}). However, icatibant reduced the increase in paw volume elicited by 1.0 mg of PGSE significantly (P<0.05, Mann-Whitney U test) to 24±4%.

The oedema was rich in plama proteins as determined by the Evans blue method. The injection of PGSE (0.1 and 1.0 mg) into the hindpaws was followed by extravasation of 42±5 and 210±34 µg Evans blue (n=4–6). While the effect of the lower dose of PGSE was not significantly reduced (28±12 µg Evans blue) by pretreatment with icatibant, the Evans blue extravasation caused by the higher dose was significantly (P<0.05, Mann-Whitney U test) inhibited by the bradykinin antagonist.

The subplantar injection of 0.1 mg of PGSE in unanaesthetized rats did not cause behavioural responses indicative of nociception (assessed by a modification of the formalin test; Cohen *et al.*, 1984). However, injection of 1.0 mg PGSE caused nociceptive responses which started about 5 min after the injection and lasted for about 10–15 min. This effect was completely (P<0.05, Mann-Whitney U test) prevented by pretreatment with icatibant.

The present results show that the extravasation of plasma and plasma proteins caused by non-algesic doses of a sonic extract from *P. gingivalis* are caused by mechanisms other than kinin release whereas the inflammatory effects of algesic doses are predominantly due to the action of kinins. The nociception elicited by the extract is solely mediated by kinins and can be prevented by icatibant. The bradykinin antagonist could thus have a potential for a clinical use against pain associated with periodontal inflammation.

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523P CHARACTERISATION OF THE PROSTAGLANDIN RECEPTORS LOCATED ON THE RABBIT ISOLATED SAPHENOUS VEIN

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The rabbit isolated saphenous vein (RbSV) is a vascular smooth muscle preparation that responds to a range of prostanoid agonists. The aim of this study was to characterise the prostanoid receptors mediating both relaxation and contraction of this tissue using a range of selective agonists and antagonists.

Isolated ring preparations from male New Zealand White rabbits (2.5-3.9 Kg) were prepared for isometric force recordings in Krebs buffer containing indomethacin (2.8µM) and GR32191B (5µM), maintained at 37°C and gassed with 95% O_2/5% CO_2. After a 60 min stabilisation period the preparations were contracted with 40mM KCl and a cumulative concentration-effect (E/[A]) curve to the standard agonist, PGE_2, was constructed. Preparations were washed and after 60 min an E/[A] curve to one of the selective test agonists was constructed. Data were fitted to a logistic function for potency information and if appropriate, to the operational model of agonism (Black & Leff, 1983) for affinity (pK_A) and efficacy (τ) estimates. The relative potencies (R.P.) for the test agonists are shown in the table (PGE_2 - p[A_{50}] = 9.38 ± 0.04, n = 15).

Agonist	R.P.	Agonist	R.P.
PGE_2	1.0	AH13205	1751 (1110 - 2760)
11-deoxy PGE_1	0.4 (0.2 - 1.1)	Sulprostone	Inactive at 3µM
11-deoxy-16,16-dimethyl PGE_2	1.2 (0.4 - 3.9)	PGD_2	244 (154 - 400)
16,16-dimethyl PGE_2	1.6 (0.4 - 5.5)	BW245C	57 (33 - 98)
Misoprostol	33 (17 - 65)	Cicaprost	195 [162 - 216]

Values are geometric means with (95% confidence limits) where n ≥ 4 or [range] where n = 3.

These data suggest that the RbSV contains prostanoid EP-, DP- and IP-receptors mediating smooth muscle relaxation. The low potency of the EP_2-receptor agonist, AH13205, and the inactivity of the EP_1/EP_3-receptor agonist, sulprostone, suggest that this is an 'atypical' EP-receptor. The putative EP_4-receptor antagonist, AH23848B, caused a rightward displacement of PGE_2 E/[A] curves yielding a pA_2 of 4.93 ± 0.08 (n = 4) which is consistent with an activity at EP_4-receptors (Louttit *et al*, 1992). The two 16,16-dimethyl substituted compounds were partial agonists at this receptor and direct model fitting yielded pK_A estimates of 8.65 ± 0.10 (n = 5) and 8.53 ± 0.16 (n = 7), and τ estimates of 1.40 ± 0.22 (n = 5) and 5.89 ± 1.40 (n = 7) respectively. Thus, the RbSV would appear to represent a reliable system for the quantitative pharmacological analysis of potential EP_4-receptor agonists and antagonists.

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524P MONOCLONAL ANTIBODIES: EFFECT OF MULTIPLE DOSING ON REDUCING IMMUNOGENICITY

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Monoclonal antibodies (MoAb) are a relatively new therapy, and are proposed as treatments for certain cancers, inflammation and immune-mediated diseases. As foreign proteins however, they raise an immune response, which leads to enhanced clearance, a shorter $t_{½}$ and reduced efficacy. This becomes more important when multiple administrations of the antibody are required. In an attempt to modulate the immunogenicity, one of these antibodies, a chimeric murine anti-murine gamma1 isotype anti-tumour necrosis factor MoAb, designated TN3 19.12 g1, was injected into rats at different doses. Following a single dose in rats, at 20, 2 or 0.2mg.kg^{-1}, the initial $t_{½}$ of the 20mg.kg^{-1} group was 7.05±.69 days. The MoAb was clearly immunogenic though, evidenced as anti-MoAb serum antibodies and enhanced drug clearance from day 10. Male Sprague-Dawley rats (24) were allocated to four dose groups to study the effect of repeat dosing over 8 weeks, all injections being given one week apart: GROUP 1: 20mg.kg^{-1}; GROUP 2: 20mg.kg^{-1} once, then 2mg.kg^{-1}; GROUP 3: 60mg.kg^{-1} once, then 20mg.kg^{-1}; GROUP 4: 60mg.kg^{-1} once, then 2mg.kg^{-1}. This dosing interval approximates to the half-life of the drug. All injections were given *i.p.*, and blood samples were taken from the dorsal tail-vein under light halothane anaesthesia. Serum drug and anti-MoAb antibody (a marker of immune response) concentrations, measured by ELISA. Samples were taken before (trough) and 24h after (peak) each injection. After the last injection, samples were taken for a further 4 weeks. To study any tolerance, after a further 4 weeks (8 weeks post-drug), rats were re-allocated at random to three groups (20, 2 or 0.2 mg.kg^{-1}) and re-injected. Blood samples were taken over 30 days, and assayed as previously. Results are shown below.

	$t_{½}$ (days)	C_o (μg.ml^{-1})	Steady state (days 49 or 50)	
			Trough (μg.ml^{-1})	Peak (μg.ml^{-1})
GROUP 1	7.10 ± 0.74	207.7 ± 14.8	136.6 ± 12.8	246.7 ± 14.6
GROUP 2	8.03 ± 1.43	167.2 ± 14.0	17.1 ± 2.66	23.0 ± 2.76
GROUP 3	5.56 ± 0.52	538.8 ± 37.1	159.8 ± 31.2	254.4 ± 39.0
GROUP 4	6.98 ± 1.10	591.6 ± 37.2	33.38 ± 5.5	41.7 ± 5.59

Table 1. Pharmacokinetic parameters of TN3 19.12 g1 in rats after repeat administration. Data are mean ± s.e.mean. Analysis was by ANOVA.

The half-lives of the drug in the circulation were not significantly different between the groups. Further, there was no detectable immune response (anti-MoAb antibody). Again, when the rats were re-injected 8 weeks later, there was still no appearance of an immune response, and the half-life of the drug in the serum had not significantly altered (5.85±.33, 6.25±1.1 and 6.67±1.72 days repectively). In conclusion, despite using an antibody which is immunogenic on single administration, tolerance to it could be achieved by a course of injections, at a number of different dosing schedules. This has important implications for designing therapeutic regimens for man.

525P EFFECTS OF N-ACETYL-5-HYDROXYTRYPTAMINE ON REGIONAL HAEMODYNAMIC RESPONSES TO CHRONIC ENDOTOXAEMIA IN CONSCIOUS RATS

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Since it is known that both constitutive and inducible nitric oxide synthase (NOS) are dependent on tetrahydrobiopterin, (BH4) whose synthesis is inhibited by N-acetyl-5-hydroxytryptamine (NAc5HT; Katoh *et al.*, 1982), we investigated the possibility that pretreatment with NAc5HT (2.5 mg kg^{-1} h^{-1}) might influence the cardiovascular changes in a model of chronic endotoxaemia in conscious rats (Gardiner *et al.*, 1992). Male, Long Evans rats (350-450g, n = 17) were anaesthetised (sodium methohexitone, 40-60 mg kg^{-1}, i.p.) and had pulsed Doppler probes and intravascular catheters implanted. Animals received 24 h infusions of either LPS (150 μg kg^{-1} h^{-1}; n = 8) or LPS together with NAc5HT (n = 9), the latter infusion beginning 24 h before LPS. After 24 h infusion of NAc5HT there were no significant changes in mean arterial blood pressure, heart rate, renal haemodynamics, or hindquarters haemodynamics. However, mesenteric flow and vascular conductance showed slight, but significant ($P < 0.05$, Friedman's test) increases (from 6.6 ± 0.5 to 7.7 ± 0.5 kHz (mean ± s.e. mean) and from 64 ± 4 to 74 ± 5 [kHz mm Hg^{-1}]10^3, respectively). Cardiovascular changes during LPS infusion in the 2 groups of rats are summarised in Table 1.

Table 1. Resting cardiovascular variables and changes during infusion of LPS or LPS + NAc5HT. Values are mean ± s.e. mean; * $P < 0.05$ versus baseline (Friedman's test); † $P < 0.05$ versus LPS alone (Mann-Whitney U test).

			Change during LPS infusion		
		Resting	**2 h**	**8 h**	**24 h**
Heart rate (beats min^{-1})		323 ± 8	18 ± 9	52 ± 6*	89 ± 12*
	+ NAc5HT	333 ± 10	24 ± 7*	48 ± 6*	67 ± 13*
Blood pressure (mm Hg)		102 ± 2	-9 ± 3*	0 ± 2	-15 ± 2*
	+ NAc5HT	105 ± 1	1 ± 2†	1 ± 2	-3 ± 2†
Renal conductance ([kHz mm Hg^{-1}]10^{-3})		67 ± 5	35 ± 6*	28 ± 3*	68 ± 10*
	+ NAc5HT	59 ± 3	32 ± 7*	26 ± 7*	33 ± 8*†
Mesenteric conductance ([kHz mm Hg^{-1}]10^{-3})		57 ± 6	-8 ± 4	-6 ±4	9 ± 3*
	+ NAc5HT	74 ± 5†	-30 ± 2*†	-21 ± 2*†	-11 ± 5†
Hindquarters conductance ([kHz mm Hg^{-1}]10^{-3})		43 ± 6	4 ± 2	4 ± 3	32 ± 6*
	+ NAc5HT	36 ± 2	5 ± 2*	-2 ± 2	15 ± 3*†

In the presence of NAc5HT, the early (at 2 h) hypotensive response to LPS was abolished, presumably due to the marked mesenteric vasoconstriction, because the renal vasodilatation was unchanged; a similar picture was seen at 8 h. After 24 h, in the presence of NAc5HT, the hypotensive effect of LPS was abolished, as was the slight mesenteric vasodilatation, and the marked renal and hindquarters vasodilatations were substantially reduced. These results are consistent with a dependence on BH4 synthesis of some, but not all, of both the early and late haemodynamic responses to LPS infusion.

This work was supported by a grant from the British Heart Foundation.

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526P EFFECTS OF DOXAZOSIN AND TERAZOSIN ON INFERIOR MESENTERIC NERVE ACTIVITY, SPONTANEOUS BLADDER CONTRACTION AND BLOOD PRESSURE IN ANAESTHETISED CATS

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The considerably reduced incidence of reflex tachycardia in hypertensive patients receiving α_1-adrenoceptor antagonists such as doxazosin can be explained by the central sympathoinhibitory action of these compounds (McCall & Humphrey,1981; Ramage, 1984;1986). The usefulness of these drugs in the treatment of urinary retention could also be due to their ability to reduce central sympathetic drive to the bladder. Therefore the effect of doxazosin and terazosin on spontaneous inferior mesenteric nerve activity as well as cardiac and renal nerve activity was investigated.

Cats (2.5 -3.5 kg), anaesthetized with α-chloralose (70 mg kg^{-1}) and pentobarbitone sodium (6 mg kg^{-1}), were artificially ventilated after neuromuscular blockade with vecuronium bromide (200 μg kg^{-1}). Simultaneous recordings were made of cardiac (CNA), renal (RNA) and inferior mesenteric nerve activity (IMNA), BP, HR and femoral arterial conductance (FAC; Ramage & Wilkinson, 1989). A saline (15-20 ml) filled balloon was inserted into the bladder from which pressure was recorded. The bladder was also cannulated. In all experiments an initial i.v. infusion of vehicle (0.04M lactic acid) was carried out 20 min before infusion of the test solution. The test solutions were, vehicle (n=5) alone, doxazosin (2 mg kg^{-1} h^{-1}; n=5) or terazosin (2 mg kg^{-1} h^{-1}; n=5). These solutions were infused over 1h. The volume of the infusion was the same for each test solution (3 ml). Comparison of changes (mean ± s.e.mean) with vehicle were made by ANOVA and analysed by least significant difference test.

Doxazosin and terazosin caused a significant ($P < 0.05$) fall in BP by 5 min of 9 ± 2 and 10 ± 9 mmHg reaching 34 ± 3 and 33 ± 4 mmHg after 60 min. This was associated with little change in HR for doxazosin while terazosin caused an initial significant increase in HR of 20 ± 3 beats min^{-1} by 5 min declining by 30 min to 1 ± 5 beats min^{-1}. This tachycardia was associated with a significant increase in CNA of 128 ± 22 %. Terazosin as well as doxazosin also caused an initial increase in RNA of 53 ± 16 and 74 ± 27%, respectively after 10 min, however only the increase caused by doxazosin was significant. Both doxazosin and terazosin caused a significant decrease in IMNA after 60 min of 41 ± 2% and 43 ± 16%. The onset of this decrease in IMNA was earlier for doxazosin (20 min) compared with terazosin (40 min). Doxazosin also differed from terazosin in that it caused an immediate significant increase in FAC while this was not observed until after 20 min of infusion of terazosin. Neither drugs caused any obvious changes in spontaneous bladder contractions or tone.

Both drugs can reduce IMNA however these results indicate that unwanted cardiovascular effects would be greater for terazosin.

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527P THE CYANINE DERIVATIVE, 1,1-DIETHYL-2,4-CYANINE (DECYNIUM 24) IS A POTENT INHIBITOR OF THE RENAL TUBULAR UPTAKE OF L-DOPA

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The present work reports on the kinetic characteristics of the tubular uptake of L-DOPA and the effects of a series of compounds which are known to interfere with the cellular uptake of a wide range of substrates; the compounds tested include monoamine uptake blockers (cocaine, corticosterone and β-estradiol), the organic anion inhibitor probenecide, the organic cation inhibitor cyanine 863 (1-ethyl-2-[(1,4-dimethyl-2-phenyl-6-pyrimidinylidene)methyl]-quinolinium) and two other cyanine derivatives, 1,1-diethyl-2,4-cyanine (decynium 24) and 1,1-diethyl-2,2-cyanine (decynium 22), the organic cation n-methyl-nicotinamide (NMN) and the uncoupling agent 2,4-dinitrophenol. Suspensions of renal tubules were obtained from male Wistar rats. The accumulation of L-DOPA in renal tubules obtained from benserazide (10 mg kg^{-1}) treated rats was found to occur through non-saturable and saturable mechanisms. The kinetics of the saturable component of L-DOPA uptake in renal tubules was as follows: V_{max}=12.9±2.2 nmol mg $protein^{-1}$ h^{-1} and K_m=279 (95% confidence limits; 139, 561) μM (n=8). The diffusion constant (K_d) of the non-saturable component for the accumulation of L-DOPA was 0.0013 (0.0011, 0.0016) μmol^{-1}. The rate of tubular uptake of L-DOPA calculated considering the rate of dopamine (DA) formation subtracted from the amount of L-DOPA entering the cells by diffusion was found to be twice that observed in preparations obtained from benserazide-treated animals; V_{max}=25.2±2.5 nmol mg $protein^{-1}$ h^{-1} and K_m=99 (52, 190) μM. This led us to hypothetsize that the rate of formation of DA, subtracted from the amount of the amine resulting from the passive diffusion of L-DOPA into the tubular cells, would represent a better estimate of L-DOPA uptake into the renal tubules and this methodology was used in experiments performed in the presence of the compounds to be tested; the concentration of L-DOPA (250 μM) used in these experiments was approximately 3-fold the expected K_m for tubular uptake. The cyanine derivatives decynium 24 and decynium 22 were found to be the most potent inhibitors of L-DOPA uptake with IC_{50} values (in μM) of 0.19 (0.07, 0.56) and 0.78 (0.29, 2.08), respectively; these two compounds were found to be more potent than cyanine 863 (IC_{50}=2.02 [1.07, 3.79]). At the highest concentration tested (50 μM), the effect of probenecide was a slight reduction in L-DOPA uptake (24% reduction). A similar effect was observed for cocaine; at 50 μM, there was a 22% non significant reduction of L-DOPA uptake. In contrast, both corticosterone and β-estradiol produced considerable inhibitory effects on the uptake of L-DOPA; the IC_{50} values for corticosterone and β-estradiol were 11.0 (3.6, 33.5) and 23.7 (9.8, 57.2) μM, respectively. NMN (50 to 2000 μM) reduced the uptake of L-DOPA by 41% at the highest concentration used, with an IC_{25} value of 106.2 (28.4, 397.2) μM. The effect of 2,4-dinitrophenol was a marked reduction on the tubular uptake of L-DOPA, with an IC_{50} value of 12.1 (4.0, 36.9) μM. The inhibitory effect of decynium 24 (0.5 μM) on the tubular uptake of L-DOPA was dependent on the pH of the incubation medium: at pH=6.5 the accumulation of L-DOPA was reduced up to 37±2% of control values, whereas at pH=7.4 and pH=8.2 the accumulation of L-DOPA was reduced up to 56±1% and 60±6%, respectively. The most potent cyanine derivative, decynium 24 (5 μM), was found not to affect the decarboxylation of L-DOPA in kidney homogenates. It is concluded that the tubular uptake of L-DOPA might be promoted through an organic cation transporter.

This study was supported by grant number PBIC/C/CEN 1139/92 from the JNICT.

528P EVIDENCE THAT PRESYNAPTIC MODULATION BY NEUROPEPTIDE Y OF INHIBITORY TRANSMISSION IN SUBMUCOUS GANGLIA OF GUINEA-PIG CAECUM IS MEDIATED BY Y_2-RECEPTORS

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Most submucous neurones of the guinea-pig caecum exhibit a) at least one cholinergic fast excitatory synaptic potential (EPSP), b) an α_2-adrenoceptor-mediated slow inhibitory synaptic potential (IPSP) and c) a non-cholinergic, slow EPSP in response to focal stimulation of internodal fibre tracts (Mihara, 1993). Caecal submucous ganglia contain numerous intraganglionic nerve fibres immunoreactive for neuropeptide tyrosine (neuropeptide Y; NPY) which selectively inhibits the slow IPSP (Cunningham et al., 1994) by a presynaptic mechanism. The aim of the present experiments was to determine the subtype of neuropeptide Y receptor mediating this inhibition.

Intracellular recordings were made from submucous ganglia of mid-caecum isolated from adult albino guinea-pigs (weighing 170-550 g) killed either by stunning or overdose with anaesthetic ether followed by exsanguination. About 85% (70/81) of neurones, had a slow IPSP evoked in response to focal stimulation with trains of five pulses at 40 Hz. The value of E_m at end of recording was -55.6 ± 1.2 mV (mean ± s.e. mean; n = 46); the amplitude of the slow IPSP in response to the stimulus train at E_m was 23.8 ± 1.5 mV (n = 39). NPY (1-300 nM) was found to shorten, then to depress or abolish reversibly the slow IPSP in 37/37 cells tested; in four experiments in which 2 - 4 concentrations of NPY were tested, the IC_{50} value for the depression of the amplitude was 9.5 ± 2.9 nM. In 16 neurones, these inhibitory effects of NPY were mimicked by peptide YY (PYY; 0.3-100 nM) and NPY13-36 (1-300 nM); [Leu^{31},Pro^{34}]-NPY was without effect at concentrations of up to 300 nM. Based on the results of 26 neurones tested, the apparent rank order of potency was PYY>NPY>NPY13-36>>[Leu^{31}, Pro^{34}]-NPY. NPY and its analogues (up to 100 or 300 nM; n = 18) had no depressant effects on the active and passive properties of the impaled neurone and did not affect cholinergic fast EPSP or non-cholinergic slow EPSPs. Furthermore, they did not alter the amplitude or time-course of either depolarizations or hyperpolarizations induced by focal application of acetylcholine (n = 2) or noradrenaline (n = 7), respectively.

It is concluded that activation of neuropeptide Y_2-receptors on noradrenergic nerve terminals in these ganglia leads to an inhibition of noradrenaline release and, therefore, to a suppression of inhibitory synaptic transmission.

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529P INHIBITORY EFFECT OF SOMATOSTATIN IN MOUSE VAS DEFERENS IS INSENSITIVE TO PERTUSSIS TOXIN TREATMENT

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Controversy exists as to whether all five of the recently cloned somatostatin receptor isoforms are negatively coupled to adenylyl cyclase via pertussis toxin-sensitive G-proteins (Kaupmann et al., 1993; Raynor et al., 1993). In this study, we have investigated the effect of pertussis toxin pretreatment on the inhibitory effect of somatostatin and the selective somatostatin agonist, seglitide, on neurogenically-mediated contraction of mouse vasa deferentia and on the negative chronotropic action of carbachol on isoprenaline stimulated mouse isolated atria. Vasa deferentia and atria were removed from either control (untreated) or pertussis toxin pre-treated mice (50μg/kg administered into a tail vein approximately 48h previously) and placed in Krebs solution (NaCl 118; $NaHCO_3$ 25; KCl 4.7; $MgSO_4.7H_2O$ 0.6; KH_2PO_4 1.2; D-Glucose 11.1; $CaCl_2.6H_2O$ 1.3 mM) bubbled with 95% O_2/5% CO_2 at 37°C and 32°C, respectively, for recording isometric tension changes and rate of beating. Vasa deferentia were stimulated electrically (0.5ms pulse width 20Hz for 1s every 30s with supramaximal current, approximately 900mA).

Somatostatin (1-300nM) and seglitide (0.1-30nM) administered non-cumulatively caused a concentration-dependent inhibition of neurogenically mediated contractile responses in the mouse vas deferens. In control preparations (i.e. in the absence of pertussis toxin) seglitide (EC_{50} = 0.61±0.03nM, maximal inhibition 56±5% n=4) was approximately twenty times more potent than somatostatin (EC_{50} = 21±9nM, maximal inhibition 60±4% n=5). There was no difference in reactivity to either seglitide (EC_{50} = 0.67±0.05nM, maximal inhibition 48±8% n=4) or somatostatin (EC_{50} = 35±13nM, maximal inhibition 59±9%, n=4) in preparations prepared from pertussis toxin pre-treated animals.

Isoprenaline (0.1μM) increased the rate of spontaneously beating atria from 253±21 to 417±24 beats/min in control atria (n=6) and from 203±22 to 460±17 beats/min (n=6) in pertussis toxin pre-treated preparations. In control atria, carbachol (10-1000nM) administered cumulatively produced a concentration dependent bradycardia (363±33 beats/min @ 1μM) whilst only a small tachycardia (23±6 beats/min @ 1μM) was seen in atria from pertussis toxin pre-treated animals.

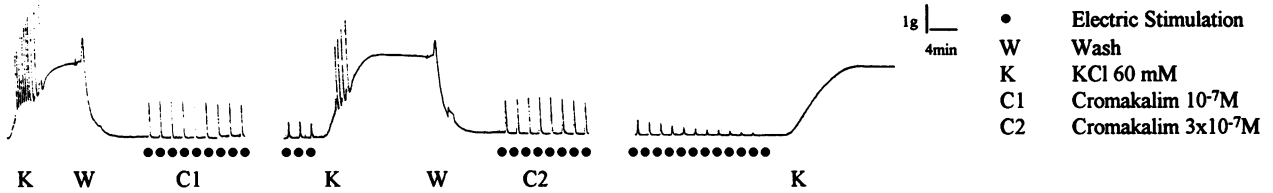
These results demonstrate that whilst muscarinic receptor activation in mouse atria can be inhibited by pertussis toxin pre-treatment, the inhibitory effect of somatostatin in mouse vas deferens is resistant to pertussis toxin. We suggest that the receptor mediating the inhibitory action of somatostatin in the mouse vas deferens is not coupled to a pertussis toxin-sensitive G-protein. Furthermore, the high potency of seglitide suggests that the receptor mediating the inhibitory effect of somatostatin in the mouse vas deferens is of the $SRIF_1$ class (Raynor and Reisine, 1992).

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530P EFFECT OF CROMAKALIM ON THE CONTRACTILITY OF THE ISOLATED HUMAN URETER

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Potassium channel openers (KCOs) have been shown to relax vascular and non vascular human smooth muscle (*Soares de Moura & Jazbik 1992; Soares de Moura et al.; 1993*). At the moment there is little information concerning the effects of KCOs in the human ureter. We have therefore investigated the effects of cromakalim, and the antagonism of its action by glibenclamide and TEA, on the human isolated ureter. Segments of human ureter obtained from kidney donors (leftovers), were carefully cleaned of any adjacent tissue and cut into rings approximately 2-4 mm long. Each ring was suspended by two stainless steel stirrups passed through its lumen and suspended in organ chambers filled with 30 ml Tyrode solution bubbled with oxygen (pH 7.4, 37°C). One stirrup was anchored inside the organ chamber. The other stirrup was connected to a force transducer for measurement of isometric force on a Nihon Koden recorder. All rings were progressively stretched to the optimal point of the length-tension curve as determined by the response to 60mM KCl and allowed to equilibrate for 60 minutes. Two platinum electrodes were placed parallel to the preparation. The ureter was stimulated, every 100s, by trains (300ms) of electric impulses (50V; 0.5ms duration; 50Hz) provided by a Grass stimulator (S48). The phasic contractions elicited by electrical stimulation of the ureter were not changed by TTX 10^{-5}M, but were completely abolished by cromakalim 10^{-7}M to 10^{-6}M (*n=12*). The inhibitory effect of cromakalim was reversed by KCl 30mM (*n=4*) and prevented by pre-treatment with glibenclamide 10^{-5}M (*n=6*) but not TEA 10^{-2}M (*n=6*). The phasic contractions induced by electrical stimulation of the ureter were not changed by glibenclamide but were enhanced by TEA. KCl 60mM induced a burst of phasic followed by a tonic contraction. Cromakalim $3x10^{-7}$M (*n=6*) abolished the phasic but not the tonic contractions induced by 60mM KCl (see figure below).



Cumulative addition of cromakalim produced a concentration-related relaxation of rings precontracted with 20mM (but not 60mM) KCl. This effect was significantly attenuated in the presence of glibenclamide. These results show that K channels are important in the modulation of the contraction of human ureter and cromakalim is a potent inhibitor of human ureter contractility, an effect probably mediated via the activation of glibenclamide-sensitive K-channels. According to the present results, we suggest that KCOs may be useful in the treatment of renal colic when hypermotility of the ureter is present.

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531P ZM172405 EVOKES SMOOTH MUSCLE RELAXATION VIA A K^+ CHANNEL OPENER MECHANISM

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ZM172405 ((S)-(-)-N-(3-chloro-4-cyanophenyl)-2-hydroxy-2-trifluoromethylpropionamide) is a novel smooth muscle relaxant. In this study we have investigated whether the mechanism which underlies the effects of this compound is associated with K^+ channel opening.

Longitudinal sections of guinea-pig hepatic portal vein or ring segments of rat aorta were suspended in Krebs' buffer at 37°C and gassed with $95\%O_2/5\%CO_2$. Changes in isometric tension were measured from a resting tension of 1g . For K^+ flux studies, changes in $^{42}K^+$ efflux from rat aortic segments loaded with this marker were measured as peak % increase in efflux.

Cromakalim, ZM172405 and ZM172406 (the (S)(+) stereo-enantiomer related to ZM172405) inhibited spontaneous mechanical activity in the guinea-pig portal vein with mean ± sem pD_2 values of 7.42±0.04 (n=73), 7.19±0.13 (n=7) and 5.94±0.22 (n=4), respectively. The actions of both cromakalim and ZM172405 were antagonised by glibenclamide (0.1-10μM) in an apparently competitive manner. For the interaction with glibenclamide, Schild analysis of concentration ratios revealed linear relationships with mean (95% confidence interval) pA_2 values of 6.69 (6.9-6.5; slope=1.13) and 6.94 (7.15-6.73; slope=1.29) for cromakalim and ZM172405, respectively. In both cases, the slope of the Schild plot was not significantly different from unity. Charybdotoxin (ChTX, 0.1μM) failed to antagonise the effects of either compound. Mean ± sem cromakalim pD_2 values were 7.13±0.3 (n=4) and 7.53±0.13 (n=4) in the presence of vehicle and ChTX (0.1μM), respectively ($p>0.05$) and mean ± sem ZM172405 pD_2 values were 7.64±0.1 (n=4) and 7.78±0.05 (n=4) in the presence of vehicle and ChTX (0.1μM), respectively ($p>0.05$). Segments of rat aorta precontracted with 20mM KCl were relaxed by ZM172405, ZM172406 and cromakalim with pD_2 values of 6.50±0.07, 5.27±0.19, and 6.57±0.12, respectively (n=4). ZM172405 produced peak increases in $^{42}K^+$ efflux of 151±16.6% ($p<0.01$) and 193.2±35.3% ($p<0.01$) when tested at 3 and 30μM, repectively whilst cromakalim (10μM) increased $^{42}K^+$ efflux by 353.4±27.4% ($p< 0.01$, n=5). In contrast, ZM172406, when tested at 10 and 100μM, increased $^{42}K^+$ efflux by only 49.2±21.3 ($p<0.05$) and 62.7±18.3% ($p<0.05$), respectively.

In this study, ZM172405, like cromakalim, produced a stereospecific, glibenclamide-sensitive, ChTX-insensitive relaxation of vascular smooth muscle. This, together with the ability of this agent to increase $^{42}K^+$ efflux from rat aorta, suggests that the effects of ZM172405 are exerted through the opening of ATP-sensitive K^+ channels. ZM172405, an anilide tertiary carbinol, represents a novel structural type of K^+ channel opener.

532P INHIBITION OF THE VASORELAXANT EFFECTS OF LEVCROMAKALIM AND P1060 BY RUBIDIUM IN HUMAN SAPHENOUS VEIN

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Potassium channel openers (KCOs) appear to open small conductance, glibenclamide-sensitive potassium channels in both arterial and venous smooth muscle (*Noack et al.*,1992; *Criddle et al.*,1994). However, recent evidence has indicated that these drugs may possess other 'non-plasmalemmal' actions. For example, in experiments in rat aorta in which the potassium ions of the physiological salt solution have been replaced by rubidium (Rb) ions the vasorelaxant effects of the KCOs are inhibited, possibly due to a blockade of membrane K-channels by Rb since these drugs can no longer elicit hyperpolarisation (*Greenwood & Weston*,1993). However, despite their inability to induce electrical changes under these conditions, KCOs can still produce a full relaxation of the precontracted rat aorta. In the present study we decided to ascertain whether such inhibitory effects of Rb also exist in human vascular smooth muscle by comparing the mechanical effects of levcromakalim (LK) and P1060 on human saphenous vein in the presence and absence of Rb.

Isometric tension was recorded from rings of saphenous vein using conventional techniques. Following stabilisation, tissues were exposed to either normal Krebs (K-Krebs) or a modified solution in which the K^+salts had been replaced by their Rb^+ equivalents (Rb-Krebs) for 30 mins (3 washes). Tone was induced in veins using 20mM KCl or 20mM RbCl as appropriate. Cumulative concentration-response curves were then constructed for each KCO in the presence or absence of Rb. In some experiments a single concentration (1μM) of KCO was used to relax precontracted veins in order to compare the duration of relaxation in the presence and absence of Rb.

Rb-Krebs produced an increase in basal tone of 0.75±0.12g (*n=12*). Comparable increases in tension were induced by 20mM KCl and 20mM RbCl (1.24±0.7g (*n=12*) and 1.65±0.28g (*n=12*), respectively). LK elicited full, concentration-dependent relaxations of precontracted veins (mean IC_{50}= 0.097µM, *n=6*) which were inhibited approximately 4-fold in Rb-Krebs (mean IC_{50}= 0.40μM, *n=6*). P1060 was a more potent relaxant of veins than LK (mean IC_{50}= 0.036μM, *n=6*) and its effects were inhibited approximately 11-fold in the presence of Rb (mean IC_{50}= 0.40µM, *n=6*). In Rb-Krebs the maximal responses of either LK or P1060 were not diminished, however, the relaxations induced by the KCOs under these conditions were transient. The relaxations induced by LK (1μM) and P1060 (1μM) in Rb-Krebs began to 'fade' after 12.43±3.07 and 6,83±0,83 mins, respectively, to new level of tone (44.02±9,1% (*n=7*) and 35.87±4,4% (*n=6*) of initial relaxation, respectively), whereas in K-Krebs these were maintained for >2 hours (*n=12*). Following this 'fade' in Rb-Krebs a higher concentration of each KCO (10μM) further relaxed the veins (*n=6*).

Our preliminary results have shown that the vasorelaxant actions of two structurally different KCOs, LK and P1060, are inhibited in the presence of Rb in human vascular smooth muscle. The effects of P1060 were inhibited to a greater extent in Rb-Krebs than those of LK, possibly indicating differences in the mode(s) of action of these KCOs. In addition, the vasorelaxant effects of LK and P1060 in Rb-Krebs in human saphenous vein were transient and are thus in agreement with previous findings in animal tissue (*Greenwood & Weston*, 1993).

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533P SR 47063, A NEW K^+ AGONIST: EFFECT ON REPERFUSION INJURY IN COMPARISON TO CROMAKALIM IN ISOLATED, PERFUSED RAT HEARTS

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Cromakalim has been suggested to reduce the extent of cellular injury injury following an ischaemic insult (Grover et al., 1989). In the present study, we have compared the ability of a new K^+ agonist, SR 47063 [4-(2-cyanimino-1,2-dihydropyrid-1-yl)-6-nitro-2,2 dimethyl-2H-1-benzopyran] to that of cromakalim, to improve the recovery of cardiac function and to reduce lactate dehydrogenase (LDH) release, measured at 25 min post-reperfusion, following 25 min global ischemia in isolated perfused rat hearts (n= 5 to 8 in each group). Hearts were perfused with 0.1, 1.0 and 3.0 µM SR 47063 and 1.0, 7.0 and 10 µM cromakalim. Left ventricular developed pressure (LVDP), dP/dtmax and heart rate (HR) were measured via a fluid-filled balloon catheter placed in the left ventricle. Coronary flow was measured manually and LDH was measured spectrophotometrically. SR 47063 at 1 and 3µM and cromakalim at 7 and 10µM improved functional recovery following global myocardial ischaemia. In addition, SR 47063 reduced significantly LDH release at 1.0 and 3.0 µM and cromakalim reduced significantly LDH release at 7µM.

Table 1 Effect of SR 47063 and cromakalim on functional recovery and enzyme leakage following myocardial ischaemia in isolated, perfused rat hearts.

	Control	SR 47063 (µM)			Cromakalim (µM)		
		0.1	1	3	1	7	10
HR (beats/ min)	252 ± 19	240 ± 19	266 ± 5	278 ± 7	248 ± 16	274 ± 5•	256 ± 7
LVDP (mmHg)	64 ± 9	50 ± 9	106 ± 5•	95 ± 2•	49 ± 14	66 ± 13	86 ± 6
HRxLVDP/1000	16 ± 2	12 ± 3	28 ± 1♦	26 ± 1•	11 ± 2	18 ± 4	22 ± 1*
dP/dt max (mmHg/s)	1320 ± 207	1170 ± 258	2640 ± 130♦	2320 ± 34•	1020 ± 282	1740 ± 303	2080 ± 128*
CF (ml/min)	12.6 ± 0.7	16.2 ± 0.8	16.2 ± 0.7•	17.3 ± 1.0	14.6 ± 0.4*	15.6 ± 0.5	17.0 ± 0.6•
LDH (IU/g wet wt)	44.9 ± 3	35.9 ± 6.6	10.9 ± 2♦	20.8 ± 6.2•	46 ± 2	32.1 ± 2.6•	36.6 ± 3

* P<0.05, • P<0.01, ♦P<0.001 in comparison to control group.

Thus, both K^+ agonists, improved significantly functional recovery and reduced enzyme leakage in isolated rat hearts following a period of global ischaemia. In addition, the effects of SR 47063 appear at a lower concentration than those of cromakalim.

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534P COMPARISON OF THE *IN VITRO* ACTIVITY OF SCA40 WITH LEVCROMAKALIM, BRL 55,834 AND SALBUTAMOL IN GUINEA-PIG AIRWAYS

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It has been proposed that the imidazopyrazine derivative SCA40 relaxes airway smooth muscle by opening charybdotoxin-sensitive potassium channels (Laurent et al, 1993). The aim of the present study was to compare the relaxant and inhibitory potency of SCA40 with those of potassium channel openers(KCOs), levcromakalim(LMK) and BRL 55,834 (Taylor et al, 1992), and the β_2-agonist, salbutamol, in guinea-pig airways. The effects of these agents were determined in the presence and absence of the potassium channel blockers, apamin or glibenclamide.

Isometric recordings were made from guinea-pig tracheal strips and bronchial rings under a resting tension of 1g. Indomethacin (2.8μM) was present throughout the experiments. Relaxant effects were assessed against KCl (20mM) and histamine (1μM) induced tone in trachea and β[Ala^8]Neurokinin A(4-10)(BANA, 1-10nM) induced tone in bronchi. In addition inhibitory potency was examined against electrically stimulated (25 volts, pulse width 0.1 ms, frequency 10 Hz, 10 s) excitatory non-adrenergic, non-cholinergic (eNANC) induced contractions in bronchi. The relaxant and inhibitory potency of these agents is expressed in the table as the mean IC_{50} value±s.e.mean.

COMPOUND	IC_{50}(μM) KCl(n)	IC_{50}(μM) His(n)	IC_{50}(μM) eNANC(n)	IC_{50}(μM) BANA(n)
SCA40	0.22±0.04(6)	0.23±0.03(13)	0.11±0.08(4)	0.30±0.08(6)
Salbutamol	0.01±0.01(7)	0.02±0.01(20)	0.01±0.01(4)	0.02±0.01(6)
LMK	0.60±0.15(6)	0.76±0.14(11)	0.17±0.03(6)	0.48±0.10(6)
BRL55,834	0.19±0.01(6)	0.31±0.05(10)	-	-

All agents relaxed the contractile responses within a similar concentration range irrespective of the spasmogen used (seeTable), as well as having similar inhibitory potency against eNANC-induced contractions. The order of potency obtained in these experiments was as follows: Salbutamol>SCA40=BRL55,834>LMK. The ATP dependent potassium channel (K_{ATP}) blocker,glibenclamide (0.3-10μM) shifted the relaxant concentration-response curves to the KCOs in a parallel rightward manner, but had no effect on either the SCA40 or the salbutamol relaxant curves. Apamin (0.1-1μM), which blocks the small conductance calcium-activated potassium channels (S_{kCa}), produced no inhibition of the relaxant effects of SCA40, LMK or salbutamol.

In summary, these experiments demonstrate that SCA40 is capable of relaxing airway smooth muscle and inhibiting eNANC-induced contractions with at least equivalent potency to the KCO compounds, LMK and BRL55,834. None of these effects of SCA40 could be attributed to the involvement of K_{ATP} or S_{kCa} channels. Experiments to define the role of charybdotoxin-sensitive potassium channels in the relaxant/inhibitory effect of SCA40 are in progress.

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535P THE EFFECTS OF SOME K$^+$-CHANNEL INHIBITORS ON THE ACTIONS OF SPASMOGENS IN GUINEA-PIG ISOLATED TRACHEALIS MUSCLE

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Various types of K+-channel have been identified in airways smooth muscle (Small *et al.*, 1993) though the roles of these channels in regulating the excitability of the muscle cells remain to be established. Boyle *et al.* (1988) showed that tetraethylammonium (TEA) and procaine (non-selective inhibitors among the various types of K+-channel) did not potentiate either acetylcholine (ACh) or histamine in contracting guinea-pig isolated trachealis muscle. In contrast, Chand *et al.* (1990) subsequently reported that TEA potentiated ACh and 5-hydroxytryptamine acting on rat isolated trachealis muscle. We have now further explored the effects of K+-channel blockade on the actions of spasmogens in airways smooth muscle using inhibitors with reported selectivity for the delayed rectifier channel (K_{dr}), the large Ca^{2+}-dependent K+-channel (BK_{Ca}) and the glibenclamide-sensitive K+-channel (K_{ATP}).

Isometric recording of tension changes showed that carbachol (CCh; 10μM - 10mM), histamine (100nM - 1mM) and KCl (5 - 120mM) each caused concentration-dependent spasm of indomethacin (2.8μM)-treated guinea-pig isolated trachealis muscle. 4-aminopyridine (4-AP; 1mM) increased basal tone and caused a fourfold leftward shift in the log concentration/effect curve for histamine (pD_2 in test tissues 5.54 ± 0.06; pD_2 in control tissues 4.93 ± 0.07; mean ± s.e. mean, n = 6). 4-AP (1mM) did not significantly alter the tissue sensitivity to KCl (pD_2 1.68 ± 0.03 and 1.61 ± 0.02 for test and control tissues respectively) but antagonised CCh (pD_2 6.19 ± 0.05 and 6.64 ± 0.04 for test and control tissues respectively). Atropine (1μM) antagonised (4.5 fold) the spasmogenic action of 4-AP (62.5μM - 8mM). In the presence of a combination of atropine (1μM) and 4-AP (1mM) the log concentration-effect curves of histamine, CCh and KCl were all significantly leftward shifted (5.8, 2.2 and 1.5 fold respectively). Glibenclamide (10μM) caused no change in baseline tone of the indomethacin-treated trachealis muscle and did not alter tissue sensitivity to CCh, histamine or KCl. Charybdotoxin (Chtx; 100nM) caused an increase in baseline tone in 7 of the 23 tissues tested. Chtx potentiated CCh (3.9 fold) and KCl (2.5 fold) but did not alter tissue sensitivity to histamine.

The failure of glibenclamide to potentiate CCh, histamine or KCl suggests that K_{ATP} plays little role in regulating the sensitivity of airways smooth muscle to spasmogens. The ability of 4-AP and Chtx to potentiate spasmogens acting on guinea-pig trachealis muscle may respectively suggest that K_{dr} and BK_{Ca} channels both play roles in modulating the excitability of the muscle cells. The sensitivity of 4-AP induced spasm to atropine supports the suggestion of Kannan *et al.* (1983) that 4-AP directly or indirectly activates muscarinic cholinoceptors on trachealis muscle cells. This effect may complicate the use of 4-AP as a tool for inhibiting K_{dr}.

L.I. was supported by Pfizer U.K. Ltd.

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536P POTASSIUM CHANNEL AND CALCIUM CHANNEL CURRENTS AND THEIR INHIBITION BY GLIBENCLAMIDE IN SMOOTH MUSCLE CELLS ISOLATED FROM GUINEA-PIG SEMINAL VESICLE

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The types of ion channel in seminal vesicle smooth muscle cells and their roles in the control of tension have not been described. We have investigated some of their properties in freshly isolated cells using the whole-cell voltage-clamp technique at room temperature. The bath solution included 130 mM NaCl and 1.5 mM $CaCl_2$ (K-current recording) or 10 mM $BaCl_2$ (Ca channel current recording). The intracellular pipette solution included 130 mM KCl or CsCl for K-current and Ba-current recordings respectively, and 3 mM ATP and 10 mM EGTA unless otherwise stated. All drugs were applied via the bath solution.

When the KCl pipette solution was used and the holding potential (HP) was -60 mV, two major types of outward current were elicited by square depolarising pulses to positive of -20 mV. At a test potential of +60 mV the first outward current had a transient appearance, reaching a peak in 5±1 ms (mean±s.e.mean, n=6) and decaying completely in 106±9 ms (n=6). The current was unaffected by 1 mM tetraethylammonium (TEA, n=6) but was inhibited by 36±9 % by 1 mM 3,4-diaminopyridine (3,4-DAP, n=5). In contrast, the second outward current was sustained for the duration of a 0.5 s-test pulse, and in some cells spontaneous transient outward currents were superimposed. This sustained current was inhibited by 87±8 % by 1 mM TEA (n=6) but 3,4-DAP (1 mM) seemed ineffective (n=6). The TEA-sensitive current component appeared to be Ca-dependent because its mean amplitude at +60 mV was 0.2±0.09 nA when there was 10 mM EGTA in the pipette (n=6) and 1.81±0.48 nA when there was 0.2 mM EGTA in the pipette (n=6). When the $BaCl_2$ bath solution and CsCl pipette solution were used, depolarisations to positive of -30 mV elicited a voltage-dependent inward current which was abolished by Cd^{2+} (100 μM) and inhibited by 79±3 % by 100 nM nifedipine when the test potential was +20 mV (n=6). We also investigated the action of the sulphonylurea glibenclamide (reviewed by Ashcroft & Ashcroft, 1992) because this compound has a surprising relaxant effect on the seminal vesicle (Sadraei & Large, unpublished). Glibenclamide (0.5 mM) inhibited the transient and sustained outward current components by 74±14 % (n=4) and 36±15 % (n=4) respectively. It also inhibited the Ba-current; 10 μM, 100 μM and 1 mM glibenclamide reduced the Ba-current by 30±6 %, 57±6 % and 77±5 % respectively (n=6), indicating an IC_{50} of about 60 μM.

These experiments suggest that at least three types of ion channel are present in seminal vesicle smooth muscle cells: A-type K channels, Ca-activated K channels and L-type Ca channels. The observed inhibitory effect of glibenclamide on Ca channel current may be a mechanism underlying its relaxant effect on the whole tissue.

HS is supported by the Iranian Ministry of Health & Medical Education and DJB by the Wellcome Trust.

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537P INTERACTIONS BETWEEN CYTOPLASMIC pH AND Ca^{2+} ON THROMBOXANE SYNTHESIS IN HUMAN PLATELETS

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Thromboxane (TX) A_2 is synthesised by aggregating platelets and promotes thrombosis. We have investigated the influence of cytoplasmic pH (pHi) on Ca^{2+} induced TXA_2 biosynthesis by human platelets. Gel filtered platelets were prepared from platelet rich plasma and suspended in HEPES buffered (pH 7.4) Tyrode's solution (NaCl 140; glucose 5.6; HEPES 5; KCl 2.8; KH_2PO_4 0.8 and $MgCl_2$ 0.4mM). Separate portions were loaded with fura-2 (Grynkiewicz *et al.*, 1985) and bis-carboxyethyl carboxy-fluorescein (BCECF, Rink *et al.*, 1986) to measure intracellular Ca^{2+} ($[Ca^{2+}]i$) and pHi respectively. Nigericin (2.7μM) was used to change pHi in platelets suspended in high K^+ Tyrode's solutions (K^+ substituted for Na^+) of varying pH. Nigericin was removed by centrifugation in the presence of forskolin (5μM) and resuspension of the platelet pellet in solution (pH 7.4) containing human serum albumin ($1gL^{-1}$ Sigma) (Ng and Dudley 1989). 2 ml aliquots were placed in a quartz cuvette in a temperature controlled chamber at 37°C in a dual excitation wavelength fluorimeter (Deltascan, PTI). Ionomycin (1200 nM) was added in the presence or absence of 1mM Ca^{2+}. pHi and $[Ca^{2+}]i$ were monitored continuously and the increase in $[Ca^{2+}]i$ above baseline determined at 60 s, at which time a 200μl portion was sampled for determination of TXA_2 production measured by radioimmunoassay of its stable hydrolysis product, TXB_2. Total protein was measured by the Lowry method. Data were analysed by paired t-test. The increase in $[Ca^{2+}]i$ and TXB_2 (mean ± s.e.mean, n = 5) in the presence of extracellular Ca^{2+} (1 mM) are tabulated:

pHi	increase in $[Ca^{2+}]i$ nM	TXB_2 (ng μg-1 protein)
6.72 ± 0.05	97 ± 18**	0.68 ± 0.2**
7.31 ± 0.02	192 ± 49	2.1 ± 0.4
7.71 ± 0.04	323 ± 56**	10.7 ± 3.3***

(**P = 0.01; ***P = 0.001)

Alkalinisation and acidification of pHi potentiated and inhibited $[Ca^{2+}]i$ and TXB_2 synthesis respectively. In the absence of $[Ca^{2+}]o$, Ca^{2+} responses to ionomycin were inhibited by 19 ± 6.5% at pH 6.8, 38 ± 11% at pH 7.4 and 65 ± 8% at pH 7.8 (each n = 4), implying an effect of pHi on Ca^{2+} entry. We conclude that pHi affects TXA_2 biosynthesis in gel filtered human platelets by influencing $[Ca^{2+}]i$.

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538P EFFECTS OF HIRULOG AND SODIUM NITROPRUSSIDE PLUS RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR ON CEREBRAL THROMBOEMBOLISM IN RABBITS

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We previously described a model of cerebral thromboembolism in rabbits in which platelet accumulation (PA) induced by thrombin (Thr) was (a) long lasting and (b) reversible by treatment with fibrinolytics such as recombinant tissue plasminogen activator (rt-PA) (May et al., 1992). The persistent PA could be due to continued generation of Thr. Secondly, SNP has been shown to prolong plasma t-PA activity in rabbits (Lidbury et al., 1990). In the present study, we have investigated (a) the effect of the Thr inhibitor, Hirulog™ (Hir) and (b) the effect of SNP on the thrombolytic action of a low dose of rt-PA against established PA responses to Thr. ^{111}In-labelled platelets were continuously monitored in the cerebral vasculature of Valium/Hypnorm treated NZW male rabbits as described elsewhere (May et al., 1992). Responses to intracarotid (i.c.) injection of Thr (90 U kg^{-1}) have been expressed as the peak % change in ^{111}In counts (within 30 min) from stable baseline values. Treatments, either by infusion or bolus dosage + infusion, were begun 30 min after injection of Thr and were continued for 45 min. Drug effects have been assessed by comparing the area under the curve (AUC) of the plots of % change against time between the start of treatment and 2 h after the end of treatment. Mean ± s.e.mean values are presented for 3-5 animals per group. The significance of differences between means was determined by analysis of variance followed by a modified t-test. In the first series of experiments, there were no significant differences between PA responses in the two groups prior to treatment (saline 96.8±11.9%; Hir 0.1mg kg^{-1} min^{-1} 95.3±8.9%). Hir treatment had no significant effect on the response measured by the AUC (saline 13282.5±1319.7; Hir 12793.1±1185.9). In the second series of experiments, peak PA responses did not differ significantly between groups (saline 91.0±6.9; SNP 30 μg kg^{-1} min^{-1} 98.3±9.8; rt-PA 0.1 μg kg^{-1} min^{-1} 90.0±17.2; SNP+rt-PA 90.3±17.5). AUC values were not significantly changed by either SNP (13828.7±1880.3) or rt-PA (8666.0±2902.4) alone compared to controls (12983.1±1100.2) whereas SNP+rt-PA significantly ($p<0.05$) reduced the response (6068.4±1035.6). These results show that (a) either continued generation of Thr plays a minor role in the prolonged PA response or is inaccessible to Hir and (b) SNP can potentiate the thrombolytic activity of rt-PA.

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539P HYDROPEROXY- AND HYDROXY-DERIVATIVES OF ARACHIDONIC ACID INHIBIT ENDOTHELIUM-DEPENDENT RELAXATION IN RABBIT ISOLATED AORTA

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During the development of atherosclerosis, endothelium-dependent vasodilation mediated by nitric oxide is impaired (Flavahan,1992). This dysfunction may result from the the accumulation of oxidised low-density lipoproteins (OXLDL) in the vessel wall. Previous studies have demonstrated that OXLDL inhibit endothelium-dependent relaxations in isolated rabbit aorta (Jacobs *et al.* 1990). In this study, the vascular effects of hydroperoxy (15-HpETE) and hydroxy-derivatives (HETE) of arachidonic acid, constituents of OXLDL, were investigated to determine whether they might contribute to the inhibition of relaxation.

The effects of these arachidonic acid derivatives on relaxations were assessed in intact aortic rings from New Zealand White rabbits as previously described (Jacobs *et al.* 1990). Tissues were precontracted with phenylephrine (PE; 0.1-0.3 μ M) and relaxations evoked with cumulative concentrations of acetylcholine (ACh; 1nM-10 μM) or glyceryl trinitrate (GTN;1 nM-1μM). After a 30 min washout period, tissues were contracted and relaxed in the presence of 15(RS)-HETE or 15(S)-HpETE, washed again and a further contraction/relaxation cycle performed to determine the reversibility of any effects.

15-HpETE (5μM) evoked contractions in intact aortic rings without active tone (0.66 ± 0.7g) and in those precontracted with PE (0.81 g ± 0.11 g). The reactivity of the tissues was unaltered by the absence of the endothelium. ACh-evoked relaxations were attenuated in the presence of 15-HpETE and 15-HETE. The maximum relaxation to ACh was reduced from 89.8 ± 2.8 % (n=4) in control tissues to 77.5 ± 2.5 % (n=4) by exposure to 15-HpETE. After washout, relaxations were restored to control values (87.0 ± 3.4 %). 15-HETE (5μM) produced a similar degree of inhibition (11.6 ± 1.7%; n=4) as its hydroperoxy precursor which was not significantly reduced in the presence of 10 μM indomethacin (11.7 % ± 0.6 %; n=4). Chelerythrine chloride, a potent protein kinase C inhibitor (Herbert et al. 1990) prevented the inhibition of relaxation by 15-HpETE, the maximum relaxation in the presence of HpETE plus chelerythrine chloride (1 μM) was 82.3 ± 3.5 % (n=7) compared with 82.9 ± 3.5 %, (n=7) in control tissues. Endothelium-independent relaxations evoked by GTN in intact and denuded tissues were attenuated in the presence of 15-HpETE. The maximum responses to GTN were reduced from 100 % to 90.3 ± 3.1 % and 90.0 ± 3.2 % in intact and denuded tissues respectively. Exposure to HETE resulted in a similar inhibition of GTN-evoked relaxations. These results demonstrate that 15-HpETE and 15-HETE cause a direct contraction of vascular smooth muscle and inhibit endothelium-dependent relaxation by a pathway(s) dependent on the activation of protein kinase which may contribute to the impairment of vasodilation in hypercholesterolaemia and atherosclerosis.

We thank British Heart Foundation and Pharmaceuticals for support.

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540P EFFECTS OF THE NO SYNTHASE INHIBITORS N$^{\omega}$–NITRO-L-ARGININE METHYL ESTER, 7-NITRO-INDAZOLE AND RELATED NITRO-INDAZOLES ON NANC RESPONSES OF GUINEA-PIG ISOLATED TAENIA CAECI

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The free radical, nitric oxide (NO), formed from L-arginine by the constitutive Ca^{2+}-dependent enzyme NO synthase (NOS), is now established as a paracrine messenger and neurotransmitter molecule (Moncada, Palmer & Higgs, 1991). The enteric nervous system generally shows a strong NOS immunoreactivity (Bredt, Hwang & Snyder, 1990), and approximately one third of cultured neurones taken from the myenteric plexus of the guinea pig taenia caeci stain NOS immunopositive (Saffrey *et al*, 1992), strongly suggesting a role for NO in this preparation. In support of this, we have previously demonstrated that the NOS inhibitor N$^{\omega}$-nitro-L-arginine (NOARG) produced a concentration-dependent partial inhibition of the non-adrenergic, non-cholinergic (NANC) relaxations of the taenia caeci (Piotrowski *et al*, 1993). The purpose of the present study was to extend the characterisation of the NO system in the guinea pig taenia caeci by examining the the effects of a number of recently reported inhibitors of NOS on this preparation.

Segments of taenia caeci 2cm long were removed from freshly killed guinea pigs (500-890g) and set up for electrical field stimulation between parallel electrodes in 10ml isolated tissue baths maintained at 37°C and supplied with Krebs solution containing atropine (10μM) and guanethidine (10μM) and bubbled with 95%O2/5%CO2. Supramaximal electrical stimulation using 1ms rectangular pulses was applied in 20Hz trains lasting 10s and repeated every 90s. Contractile activity of the preparations was recorded isotonically using a resting tension of 0.5g. The NANC relaxations are expressed as means$\pm$sem of control relaxations.

Under these conditions, the NOS inhibitor N$^{\omega}$-nitro-L-arginine methyl ester (NAME)(Palacios *et al*, 1989) produced a concentration-dependent inhibition of electrically-evoked NANC relaxations with responses of 57.2$\pm$5.1% corresponding to a maximal effect and an IC$_{50}$ for NAME of 7.4$\pm$2.2μM (n=6). The effects of 10μM NAME 89.1$\pm$2.0%* were reversed with 1mM L-arginine (97.0$\pm$1.1%‡, n=5). NANC relaxations appeared to be unaffected by the cerebellar neuronal NOS inhibitors 5-, 6-, and 7-nitro-indazole (5-, 6- & 7-NI) (Babbedge *et al*, 1993) over the concentration range 100nM-10μM. At a drug concentration of 10μM, NANC relaxations were: 5-NI 95.5$\pm$1.6%, 6-NI 99.2$\pm$4.3%, 7-NI 97.0$\pm$1.9% (n=4). In tissues exposed to 10μM of each of these inhibitors, NOARG (10μM), added subsequently, was still able to produce an inhibition of the observed relaxations: post 5-NI 78.8$\pm$3.6%*, 6-NI 85.9$\pm$5.9%*, 7-NI 79.2$\pm$8.7%*(n=4), NOARG alone 81.4$\pm$3.3%*(n=6).

The results support the previous findings that NO may have a role in mediating a component of the electrically-evoked NANC relaxations of the guinea pig taenia caeci. However, the nitrergic system in this tissue appears to differ from that found in the central nervous system.
(*=$p<0.05$, compared to pre-drug controls, ‡=$p<0.05$ compared to responses in presence of NAME alone, Student's t-test)
This work was supported by NESCOT.

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541P ACTIVATION OF SOLUBLE GUANYLYL CYCLASE BY A FACTOR OTHER THAN NITRIC OXIDE OR CARBON MONOXIDE CONTRIBUTES TO THE VASCULAR HYPOREACTIVITY TO ENDOTOXIN IN THE RAT AORTA

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The vascular hyporeactivity to various vasoconstrictors caused by endotoxin (lipopolysaccharide, LPS) is associated with an increase in cyclic GMP within the vascular smooth muscle (Fleming *et al.*, 1991). Here, we investigate the relative importance of (i) the activation of soluble guanylyl cyclase (sGC), and (ii) of potential endogenous mediators of sGC activation, in the vascular hyporeactivity to noradrenaline (NA) caused by LPS in the thoracic aorta of the rat *ex vivo*. At 3 h after an i.v. injection of E. coli LPS (10 mg kg^{-1}) to anaesthetized (thiopentone sodium, 120 mg kg^{-1} i.p.) rats, there was a significant reduction in the contractile responses elicited by NA (10^{-9}-10^{-6} M) when measured in endothelium-denuded aortic rings *ex vivo* (as previously described; Thiemermann *et al.*, 1993). Methylene blue (10^{-5} M for 20 min) or LY-83583 (10^{-5} M for 20 min), inhibitors of sGC, completely reversed the vascular hyporeactivity to NA caused by LPS, whereas inhibition of nitric oxide (NO) synthase activity with N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME; 3x10^{-4} M for 20 min) caused only a partial restoration of these responses. Neither zinc protoporphyrin-IX (Zn-PP-IX, 10^{-5} M for 20 min), an inhibitor of haem-oxygenase I (Verma *et al.*, 1993), nor the cyclooxygenase inhibitor indomethacin (5x10^{-6} M for 30 min), restored the vasoconstrictor responses to NA in aortic rings from LPS-treated rats. None of the agents tested affected the contractions elicited by NA in endothelium-denuded rings obtained from sham-operated rats (control) (Table 1).

Table 1	Control (n=8)		LPS (n=8)	
Treatment	before	after	before	after
Methylene blue	2.38±0.11 g	2.29±0.16 g	1.29±0.11 g	2.23±0.10** g
LY-83583	2.44±0.14 g	2.40±0.13 g	1.30±0.11 g	2.35±0.07** g
L-NAME	2.40±0.13 g	2.34±0.09 g	1.34±0.10 g	1.94±0.11* g
Zn-PP-IX	2.41±0.14 g	2.38±0.08 g	1.32±0.11 g	1.24±0.08 g

*Table 1. Contractile responses elicited by NA (10^{-7} M) in rat aortic rings obtained from rats treated with vehicle (control) or LPS (10 mg kg^{-1}, i.v. for 3 h) before and after treatment. ** $P<0.01$ & * $P<0.05$ versus before; Student's paired t-test.*

Thus, activation of sGC in the vascular smooth muscle accounts for the vascular hyporeactivity to NA by LPS. In addition, we demonstrate that activation of sGC by a factor other than nitric oxide or carbon monoxide contributes to the vascular hyporeactivity by endotoxin in the rat aorta. *(This work was supported by a grant from Glaxo Group Research Ltd. C.C.W. is supported by the NDMC of Taiwan, ROC. C.S. is a fellow of Lloyd's of London Tercentenary Foundation.)*

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542P FORSKOLIN POTENTIATES BOTH SNP- AND ANP-STIMULATED cGMP ACCUMULATION IN PORCINE ISOLATED PALMAR LATERAL VEIN

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Nitric oxide, a potent vasodilator, is known to stimulate soluble guanylyl cyclase, resulting in an increase in intracellular cGMP. The effects of cGMP are mediated through several mechanisms, one of which is activation of cGMP-dependent protein kinases. Using a ^{3}H-guanine prelabelling technique to study cGMP levels in guinea-pig cerebellar slices, Hernandez et al. (1994) reported interactions between cGMP and cAMP. The aim of this study was to adapt the prelabelling technique for use with vascular smooth muscle and to then study any interaction of forskolin, an adenylyl cyclase activator, on cGMP stimulation via soluble and particulate guanylyl cyclase activation in the porcine isolated palmar lateral vein.

Porcine palmar lateral veins were dissected from fore-trotters and placed in Kreb's solution. Veins were cut into 5mm sections and incubated in Kreb's solution at 37°C for 60 min in a shaking water bath. The tissues were then incubated for a further 60 min at 37°C in the presence of ^{3}H-guanine (specific activity = 8.75Ci/mmol; 20μCi/10ml). Tissues were washed by resuspension 3 times and transferred to incubation tubes (1 per tube) with an assay volume of 300μl; each experiment was carried out in quadruplicate. 20 min was allowed for equilibrium to be reached (either in the presence or absence of either 3-isobutyl-1-methylxanthine (IBMX; 10^{-3}M) or rolipram (10^{-5}M)), and the agonists sodium nitroprusside (SNP; 10^{-5}- 10^{-3}M) and atrial natriuretic peptide (ANP; 10^{-8} - 10^{-6}M) added for a period of 5 min. In studies using the adenylyl cyclase activator, forskolin ($3x10^{-5}$M), this was added 10 min prior to the addition of either SNP or ANP. 5 min after the addition of either SNP or ANP the incubation was stopped by the addition of 200μl 1M HCl. ^{3}H-cGMP was separated from ^{3}H-guanine and other ^{3}H-products using sequential Dowex/alumina chromatography (based on Salomon et al., 1974). Radioactivity was determined using liquid scintillation counting.

Both ANP and SNP produced concentration-dependent increases in ^{3}H-cGMP in the presence of IBMX (10^{-3}M) in porcine isolated palmar lateral vein. Forskolin ($3x10^{-5}$M) increased ^{3}H-cGMP levels to 118.5±8.7% of basal values (n=8). In the absence of IBMX, forskolin significantly increased both the SNP and ANP-stimulated cGMP accumulation at all concentrations of SNP and ANP used. For example, effects at the maximal SNP (10^{-3}M) and ANP (10^{-6}M) concentrations were: SNP: 154.7±15.4% of basal; SNP+Forskolin: 191.3±14.8% of basal ($p<0.05$, n=4); ANP: 161.4±17.4% of basal; ANP+Forskolin: 220.0±20.0% of basal ($p<0.05$, n=4). The cAMP selective phosphodiesterase inhibitor, rolipram (10^{-5}M), had no effect on basal or SNP-stimulated cGMP levels, however the combination of forskolin and rolipram produced an increase in the basal (158.7±27.1% of basal) and SNP-stimulated cGMP accumulation (SNP: 165.3±8.7% of basal; SNP+Forskolin+Rolipram: 510.7±64.8% of basal; $p<0.05$, n=5), greater than either forskolin or rolipram alone.

This study shows that it is possible to measure and pharmacologically manipulate cGMP using a ^{3}H-guanine prelabelling technique in porcine isolated palmar lateral vein. Stimulation of ^{3}H-cGMP levels by both SNP and ANP indicates the presence of both soluble and particulate guanylyl cyclase. The ability of forskolin to potentiate both the SNP- and ANP-stimulated ^{3}H-cGMP accumulation indicates an interaction between the two second messengers and suggests that cAMP and cGMP might produce vasodilatation via a common mechanism.

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543P EFFECT OF L-NAME ON SEXUAL BEHAVIOUR IN THE MALE RAT

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Nitric oxide seems to play a very important role in penile erection (Bush et al., 1992). The present study was performed to determine the effect of chronic inhibition of NO synthesis, induced by L-NAME, on sexual behaviour of the male rat. Male Wistar rats (250-290g) were divided in two groups. Males were placed in the observation cages a minimum of 10 min before a receptive/proceptive female was introduced and observations begun. The following parameters were recorded: Mount latency (**ML**), the time (s) from the introduction of the female to the first mount; intromission latency (**IL**), the time (s) from the introduction of the female to the first intromission; number of mounts (**NM**) prior to ejaculation; number of intromissions (**NI**) before ejaculation; ejaculation latency (**EL**), time (min) from the first intromission to ejaculation; postejaculatory interval (**PEI**), time (min) from ejaculation to the first intromission of the second copulatory series; copulatory efficiency (**CE**), number of intromissions, (multiplied per 100) divided by the total number of mounts with or without penile insertion; intercopulatory interval (**ICI**), the time (s) between intromissions; copulatory rate (**CR**), percentage of rats that complete a copulatory series. Mating behaviour tests were performed 15 days before and 1, 7, 14, 21 and 28 days during treatment with L-NAME 70 mg/Kg/day (n=10, one rat died after 15 days of L-NAME treatment) and L-arginine 70mg/Kg/day (n=10) in drinking water. Results (mean±sem) are expressed in the following table.

Day		*ML(s)*		*IL(s)*		*NM*		*NI*		*EL(min)*		*PEI(m)*		*CE(%)*		*ICI(s)*		*CR(%)*	
		ARG	NAME	ARG	NAME	ARG	NAME	ARG	NAME	ARG	NAME	ARG	NAME	ARG	NAME	ARG	NAME	ARG	NAME
-15	M	38.5	46.5	66.0	74.5	17.0	18.6	14.4	15.8	8.48	10.63	5.95	6.05	45.88	45.38	35.08	40.19	100	100
	sem	7.78	10.5	12.5	19.0	1.33	0.92	1.15	1.33	0.74	0.88	0.27	0.25	0.40	1.66	4.66	4.91		
01	M	15.5	13.5	31.5	30.5	15.5	6.6	13.0	14.4	5.39	7.95	4.87	4.48	45.43	46.81	31.72	30.09	100	100
	sem	4.11	5.77	8.33	7.73	1.51	1.4	1.32	0.99	0.98	0.72	0.19	0.19	0.99	2.32	4.66	2.44		
07	M	11.5	8.0	16.5	16.0	12.0	15.4	11.4	10.1	7.07	5.36	3,72	* 4.35	50.26	* 39.49	24.81	8.62	100	100
	sem	2.11	0.82	2.59	2.77	1.71	1.73	0.99	1.13	0.51	0.84	0.23	0.19	3.06	1.57	2.96	2.77		
14	M	11.11	17.0	19.0	26.0	7.0	* 14.3	8.56	9.2	3.74	5.18	3.8	* 4.75	54.45	* 37.66	20.52	31.29	100	100
	sem	1.62	5.54	4.47	8.52	1.14	2.1	1.09	1.61	0.94	0.79	0.26	0.19	3.92	2.63	1.92	2.85		
21	M	7.5	13.33	10.0	106.11	6.7	* 20.89	8.0	* 4.11	3 .59	5.04	4.13	4.57	57.82	* 22.35	22.62	67.74	100	* 67.6
	sem	1.54	2.5	1.67	50.71	1.44	4.42	0.71	1.01	0.76	1.89	0.20	0.29	4.56	5.73	3.65	39.55		
28	M	6.5	* 41.67	8.5	* 518.33	5.2	* 29.0	7.7	* 1.78	3.13	* 8.5	4.84	* 5.88	59.14	* 5.75	21.57	79.50	100	* 22.2
	sem	0.76	17.4	1.98	151.04	0.55	3.58	0.75	1.01	0.40	1.0	0.28	0.21	2.04	3.89	1.81	34.50		

* $p< 0.05$ between L-NAME and Arg groups

The present results demonstrate that chronic treatment with L-NAME affects the copulatory behaviour of sexually active male rats. According to our finds we suggest that chronic inhibition of NO synthesis may induce an inhibition of penile erection since the number of intromissions and copulatory efficiency were significantly reduced and intromission latency was prolonged by L-NAME treatment. Sexual drive seems not to be inhibited by L-NAME since the number of mounts were not reduced.

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544P ENHANCED RENAL DOPAMINERGIC ACTIVITY DURING LONG-TERM NITRIC OXIDE SYNTHASE INHIBITION IN THE RAT

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The present work reports on the changes in the urinary excretion of L-DOPA, dopamine (DA) and amine metabolites during long-term adminstration of N^G-nitro-L-arginine methyl ester (L-NAME; 50 μg ml^{-1} in drinking water) to rats. Aromatic L-amino acid decarboxylase (AAAD) activity in renal tissues and the ability of newly-formed DA to leave the cellular compartment where the synthesis of the amine has occurred were also determined. Twenty four hours after exposure to L-NAME, mean blood pressure (measured by the tail cuff method) was found to be increased by 20 mm Hg; heart rate slightly decreased. During the next 13 days both systolic and diastolic blood pressure increased progressively reaching 170±3 and 116±3 mm Hg, respectively. Baseline urinary excretion of L-DOPA, DA, dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and homovanillic acid (HVA) during the four day period of stabilization averaged 4.4±0.5, 13.8±0.3, 37.4±0.8, 180.0±2.7 and 206.1±6.7 nmol day^{-1}, respectively. The urinary excretion of L-DOPA, DA and DOPAC, but not that of 3-MT and HVA, were found to be increased from day 8 of L-NAME administration onwards (L-DOPA, from 15% to 67%; DA, from 14% to 61% increase; DOPAC, from 11% to 66% increase). No changes were observed in the urinary excretion of Na^+, K^+ and creatinine. Concentrations in plasma (in pmol ml^{-1}) of L-DOPA (8±2 vs 16±1), noradrenaline (2.4±0.5 vs 8.4±1.3), adrenaline (1.9±0.4 vs 9.1±0.7) and dihydroxyphenylglicol (DPHG) (3.0±0.3 vs 7.9±1.4) were found to be increased in L-NAME-treated rats, when compared to non-treated animals. AAAD activity determined in homogenates of renal tubules (V_{max}, in nmol mg $protein^{-1}h^{-1}$; K_m, in μM) was found to be in rats given L-NAME for 14 days (V_{max}=25±2; K_m=72±10) significantly ($P<0.01$) higher than that occurring in control rats (V_{max}=14±1; K_m=63±7), rats given L-NAME for 7 days (V_{max}=15±1; K_m=69±5) and rats given L-NAME plus L-arginine (1 mg ml^{-1}) (V_{max}=13±1; K_m=60±3) for 14 days. In experiments carried out in kidney slices, a considerable amount of the total DA formed from added L-DOPA was found to escape into the incubation medium. The application of the Michaelis-Menten equation to the net transport of newly-formed DA has allowed the identification of a saturable and a non-saturable component. No significant differences on the diffusional rate of transfer (K_d=0.14±0.02 $\mu moles^{-1}$) were observed between the four experimental groups. However, the saturable outward transfer of DA (V_{max}, in μmol mg $protein^{-1}h^{-1}$; K_m, in μM) in control animals (V_{max}=2.3±0.2; K_m=568±67) was higher than that in rats treated with L-NAME for 14 days (V_{max}=0.8±0.02; K_m=241±21), but similar to that observed in rats given L-NAME for 7 days (V_{max}=3.9±0.5; K_m=1006±32) and rats receiving L-NAME plus L-arginine (V_{max}=2.4±0.2; K_m=618±61). In conclusion, the present studies show that the hypertensive response resulting from the long-term administration of L-NAME is accompanied by an increased urinary excretion of DA, which appears to result from an enhanced activity of renal AAAD and increase in the delivery of L-DOPA to renal tissues. The result that the increased AAAD activity can be reversed by the administration of L-arginine to L-NAME-treated rats favours the view that the adaptative response which results in an enhanced AAAD activity probably involves a decrease in the generation of nitric oxide.

This study was supported by grants number PBIC/C/CEN 1139/92 and PBIC/C/CEN 1133/92 from the JNICT.

545P ALLOSTERIC REGULATION OF THE RAT D_2 DOPAMINE RECEPTOR BY AMILORIDE ANALOGUES

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Antagonist binding to D_2 dopamine receptors expressed in mammalian cells has been shown to be allosterically modulated by sodium and hydrogen ions, and by amiloride, a compound that is thought to interact with sodium binding sites (Neve *et al*, 1990; Neve, 1991). To investigate allosteric regulation of ligand binding to D_2 dopamine receptors, we have studied the effects of amiloride and a number of amiloride analogues on the dissociation kinetics of the antagonist [^{3}H]spiperone. Amiloride accelerates [^{3}H]spiperone dissociation from the D_2 dopamine receptor, expressed in LTK59 cells (Castro & Strange, 1993), with an EC50 of 199 ± 43μM (mean ± s.e., n=3). At a concentration of 1mM, the drug induces a 2.7-fold acceleration of dissociation over the control rate (0.0114 ± 0.0007 min^{-1}).

Three higher potency analogues have been identified; 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIA), 5-(*N*-methyl-*N*-isobutyl)amiloride (MIA) and 5-(*N*,*N*-hexamethylene)amiloride (HMA). They accelerate [^{3}H]spiperone dissociation with EC50 values of 20 ± 5μM (EIA), 13 ± 1μM (MIA) and 10 ± 2μM (HMA) (means ± s.e., n=3). As well as acting at a lower concentration, these analogues also produce a greater acceleration of the dissociation of [^{3}H]spiperone; at 300μM a 52-, 79-, and 71-fold increase in the [^{3}H]spiperone dissociation rate was observed for EIA, MIA and HMA respectively.

These higher potency analogues of amiloride should prove useful for elucidating the mechanism of allosteric regulation of the D_2 dopamine receptor.

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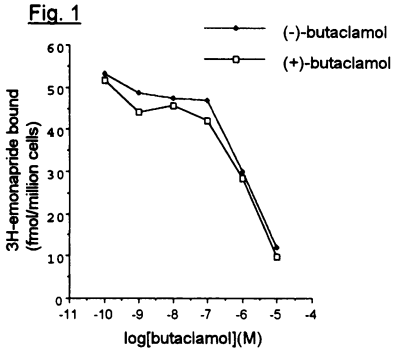
546P INVESTIGATION OF DOPAMINE RECEPTORS ON THE PERIPHERAL BLOOD LYMPHOCYTES OF HEALTHY VOLUNTEERS

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There has been a longstanding controversy over the existence and nature of binding sites for dopamine and dopamine antagonists such as spiperone, on peripheral blood lymphocytes (PBL), with such sites reported at levels from 0.5 fmol/10^6 cells to 30 fmol/10^6 cells. We used reverse-transcription and polymerase chain reaction (RT-PCR) to investigate the presence of mRNA for dopamine D_2 and D_3 receptors in PBL of healthy volunteers. We then used [^{3}H]emonapride, a high affinity ligand for D_2-like receptors (D_2, D_3 and D_4) to investigate the presence of such receptors on PBL of normal volunteers, and to investigate whether [^{3}H]emonapride was a better ligand to define the high affinity sites reported for [^{3}H]spiperone.

Using "nested PCR" , where products of a first amplification with one set of primers are amplified again with a second set of primers, we were unable to detect any D_2 sequences in mRNA of PBL. Both sets of primers were specific to the D_2 sequence and would be expected to pick up very low levels of D_2 mRNA. Using primers specific to D_3 we were able to confirm previous reports that D_3 mRNA is present in human PBL. RT-PCR is, however, unable to quantify the mRNA levels or assess its functional significance.We therefore examined binding of [^{3}H]emonapride to PBL and the ability of (+)- and (-)-butaclamol, haloperidol and (+)- and (-)-sulpiride to compete with [^{3}H]emonapride.

Fig. 1



(+)- and (-)-sulpiride were both unable to compete significantly even at 10^{-5} M. For both butaclamol and haloperidol there was inhibition of binding of [^{3}H]emonapride in the micromolar range, but with no stereoselectivity for the enantiomers of butaclamol. This was true at concentrations of [^{3}H]emonapride near the K_D values reported for D_2 like receptors (0.5 nM) and concentrations several fold greater (ie 4nM). An example of the competition curves is shown for (+)- and (-)-butaclamol in Fig. 1. For haloperidol, IC_{50}=1μM, n=3. The properties of the [^{3}H]emonapride binding in PBL are not consistent with those of typical D_2-like receptors.

We also studied the stereoselectivity of competition by (+)- and (-)-butaclamol for [^{3}H]7-OH DPAT binding to PBL. [^{3}H]7-OH DPAT is a selective ligand for the D_3 receptor. Again we saw no stereoselectivity for competition, confirming that there are no D_3 receptors expressed on PBL with the properties expected.

We conclude that D_3 but not D_2 mRNA is expressed in human PBL. However, we were unable to show that any of the D_2-like receptors are functionally expressed in PBL.

JMV is a Wellcome Clinical Research Fellow

547P THE ROLE OF ACETYLCHOLINE AND PROSTAGLANDIN RELEASE IN THE ACTION OF HYDROGEN PEROXIDE ON GUINEA-PIG DISTAL COLON

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Mucosal levels of reactive oxygen metabolites, including hydrogen peroxide (H_2O_2), are elevated in the inflammatory condition of the bowel, ulcerative colitis (Van der Vliet & Bast, 1992). Previously, we have shown H_2O_2 to be a spasmogen of guinea-pig distal colonic smooth muscle by a mechanism partially sensitive to tetrodotoxin (TTx) (Piper et al., 1993). The aim of this study was to determine the nature of the neurotransmitter released and to determine if release of prostaglandins are also involved.

Strips of distal colon from Tricolour guinea-pigs (250-350 g) were set up in tissue baths for recording of isometric tension from either the longitudinal or circular muscle axis. In longitudinally-orientated strips H_2O_2 (500 μM) evoked a transient spasm of which was partially inhibited by indomethacin (2.8 μM) (mean % spasm: Initial 100 ± 0; indomethacin-incubated 47.5 ± 8.1; vehicle-incubated 123.2 ± 7.1, $P < 0.01$, n=6). In the presence of indomethacin (2.8 μM) the remaining spasm evoked by H_2O_2 (500 μM) was abolished by either TTx (1 μM) (mean % spasm: Initial 100 ± 0; TTx-incubated 1.5 ± 3; vehicle-incubated 116.2 ± 10, $P < 0.01$, n=6) or atropine (1 μM) (mean % spasm: Initial 100 ± 0; atropine-incubated 19.6 ± 6.0; vehicle-incubated 113.6 ± 18.9, $P < 0.01$, n=5). H_2O_2 (500 μM)-induced spasm was not inhibited by the prostaglandin EP_1 receptor antagonist AH 6809 (2 μM) (mean % spasm: Initial 100 ± 0; AH 6809-incubated 80 ± 8.4; vehicle-incubated 82.9 ± 10.9, $P > 0.05$, n=10)

H_2O_2 (500 μM) was without effect on circularly-orientated strips. However, in the presence of indomethacin (2.8 μM), H_2O_2 (500 μM) evoked a transient spasm which was abolished by either TTx (1 μM) (mean % spasm: Initial 100 ± 0; TTx-incubated -2.5 ± 3.8; vehicle-incubated 113.6 ± 10.4, $P < 0.01$, n=6) or atropine (1 μM) (mean % spasm: Initial 100 ± 0; atropine-incubated -8.2 ± 4.9; vehicle-incubated 59.2 ± 14.9, $P < 0.01$, n=4). In the presence of AH 6809(2 μM), H_2O_2 (500 μM) evoked a transient spasm which was abolished by atropine (1 μM). Incubation of circularly-orientated strips with prostaglandin E_2 (PGE_2, 1 μM) resulted in a rightward shift of the concentration-effect curve to carbachol (mean -log EC_{50}: Initial 6.73 ± 0.07; PGE_2-incubated 5.84 ± 0.06, $P < 0.01$, n=6).

These results indicate that the action of H_2O_2 in guinea-pig distal colon involves two mechanisms:-
(1) release of acetylcholine from nerve terminals and (2) generation of prostaglandins. In circularly-orientated strips PGE_2 appears to be involved in the action of H_2O_2 and could explain the failure to observe a spasmogenic effect of H_2O_2 in the absence of either indomethacin or AH 6809. In longitudinally-orientated muscle PGE_2 does not appear to be involved and the exact nature of the indomethacin-sensitive component remains to be determined.

Supported by the National Association of Colitis & Crohns' Disease. AH 6809 was a kind gift from Glaxo Group Research.

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548P EFFECTS INDUCED BY LINEAR PEPTIDE ANTAGONISTS SELECTIVE FOR ET_A RECEPTORS: A STUDY IN DIFFERENT SMOOTH MUSCLE PREPARATIONS

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Endothelins induce contractions through ETA-R and ETB-R in the guinea-pig trachea (Battistini et al., 1994a), through ETB-R in the rat stomach (Warner et al., 1993), through ETA-R in the rat thoracic aorta and through mixed ET-R (ETB-R + ETX-R) in the guinea-pig gallbladder. Here, we have studied the agonist activities of endothelin selective and non-selective ETA/ETB receptor antagonists in isolated tissues. We used BQ-123 (a cyclic pentapeptide antagonist selective for ETA-R; cyclo[-D-Trp-D-Asp-L-Pro-D-Val-L-Leu-]; Ihara et al., 1992), BQ-610 (homopiperidinyl-carbonyl-L-Leu-D-Trp(CHO)-D-Trp-OH; Ishikawa et al., 1992) or FR139317 ((N, N-hexamethylene) carbomoyl-L-Leu-D-Trp(Me)-D-(2-pyridyl)Ala-OH; Sogabe et al., 1993), two linear peptide antagonists selective for ETA-R, and PD 145065 (Ac-D-Bhg-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp; Cody et al., 1993), a linear hexapeptide antagonist non-selective for ETA/ETB-R.

Male DH guinea-pigs (300-350 g) were killed by cervical dislocation. Male Wistar rats (250-300 g) were killed with thiopentone sodium (Sagatal, 120 mg kg^{-1} i.p.). The guinea-pig trachea and gallbladder, the rat aorta and stomach were rapidly excised, placed in warmed (37°C) and oxygenated (95% O_2 : 5% CO_2) Krebs-Henseleit buffer solution (pH 7.4) and trimmed free of connective tissue and fat. Rings were prepared from the trachea (-EpC) and the thoracic aorta (-EC) while strips were prepared from the gallbladder and the fundal part of the stomach. The tissues were mounted in 10 ml organ baths under tensions of 1.0 g and connected to isometric transducers (Hugo Sachs K30, Germany) coupled to a chart recorder (Graphtec WR3310, Japan). After equilibration for 60 min (with washing every 15 min), the tissues were contracted three times with 80 mM KCl, with a 30 min washout period between each contraction. Cumulative concentrations (0.1-100 μM) of antagonists were added to the tissues. Only one antagonist was tested in each preparation. The responses were standardized as the percentage of the mean contraction evoked by 80 mM KCl.

BQ-123 or PD145065 did not affect the basal tone of any of the preparations studied (n=3-6). Similarly, FR139317 or BQ-610 had no effect in the rat thoracic aorta, the rat stomach fundus or the guinea-pig trachea (n=3-6). Conversely, FR139317 or BQ-610 both induced concentration-dependent contractions of the guinea-pig isolated gallbladder (see Table 1).

Table 1. Contractile effect of ET antagonists

antagonists	n	guinea-pig gallbladder							
concentrations		0.01	0.03	0.1	0.3	1	3	10	30 μM
BQ-123	3	0	0	0	0	1	1	1	3
PD145065	3	0	0	1	1	2	2	3	4
FR139317	14	0.6	3.1	8.8	22	41	63	83	92
BQ-610	8	0.1	1.8	29	49	64	75	78	74

Thus, linear peptide antagonists selective for ETA-R such as the FR139317 or BQ-610 are agonists in the guinea-pig gallbladder. These compounds should therefore be used with caution. It remains to be determined if other linear peptide antagonists selective for ETA-R such as the BQ-485 (perhydroazepin-1-yl-L-Leucyl-D-Tryptophanyl-D-Trp-OH; Itoh et al., 1993) also produce these effects. **We thank Parke-Davis for a grant. BB is a recipient of Research Fellowship Awards from the National Heart and Stroke Foundation of Canada and the FCAR du Québec.**

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549P EVIDENCE FOR SIGNALLING BY ENDOTHELIN ET-A AND ET-B RECEPTORS IN BOVINE PULMONARY ARTERY SMOOTH MUSCLE CELLS

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The vasoconstrictor peptide endothelin (ET) was first discovered as a secretory product of cultured endothelial cells (1). At least two subtypes of ET receptors exist, (designated ET-A and the ET-B), both of which are coupled via G-proteins to their intracellular effectors. In this study, smooth muscle cells were cultured from bovine pulmonary artery by the explant method and were subcultured following harvesting using a cell scraper. Cells of passage numbers 3-10 were used in experiments. Displacement analysis of ET receptor binding was performed by incubating cells with ^{125}I-ET-1 (10pM) in the presence of increasing concentrations of unlabelled ET-1, ET-3 or sarafotoxin 6C for 2hr at 37^{0}C. Total inositol phosphate formation was measured by exposing [^{3}H]-inositol-loaded cells (4μCi/ml for 48 hr) to ET-1 at 37^{0}C in the presence of 20mM Li^+. Adenylyl cyclase activity was measured in plasma membranes prepared from cells, according to the method of Johnson et al.(2) except that assay concentrations of Mg^{++} and Na^+ were 2 and 100 mM respectively.

Analysis of displacement of specifically bound ^{125}I-ET-1 by the ET-B receptor selective ligands ET-3 and sarafotoxin 6C yielded biphasic curves (Hill slope < 1) indicating the presence of two receptor populations, while ET-1 produced a monophasic displacement curve (Hill slope not significantly different from unity). ET-1 caused a time- and concentration-dependent increase in total inositol phosphate formation (1.45 +/- 0.04 fold with 10nM ET-1 at 10 min, n=3) which was significantly inhibited ($p < 0.05$) by the ET-A receptor antagonist FR139317 (1μM). Basal and forskolin (10μM) stimulated adenylyl cyclase activities were significantly inhibited by ET-1(0.1μM) to 57.2 +/- 8.53 % and 75.1 +/- 6.54 % of control activity respectively ($p < 0.05$, n=6-9 observations from 3 separate experiments) and this effect was reversed by the ET-B receptor antagonist IRL1038 (10μM) but was unaltered by FR139317 (10μM).

These results indicate that ET-A and ET-B receptor subtypes present on bovine pulmonary artery smooth muscle cells are coupled to distinct signal transduction cascades, the stimulation of inositol phosphate formation and the inhibition of adenylyl cylcase activity respectively.

This work was supported by the Irish Heart Foundation.

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550P ARE DIFFERENT ANTAGONIST pA_2 VALUES AGAINST ENDOTHELIN-1 AND ENDOTHELIN-3 DUE TO DIFFERENT ET_A RECEPTOR SUBTYPES IN GUINEA-PIG, RAT AND RABBIT VASCULAR TISSUE?

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It has been reported that the endothelin ET_A-receptor antagonist, BQ123 (Ihara et al., 1992a) has different pA_2 values against the contractile effects of endothelin-3 (ET-3) and endothelin-1 (ET-1) in the rat aorta (Sumner et al.,1992; Kengatharan et al.,1993). We have therefore investigated the pA_2 values of this antagonist against ET-1 and ET-3 in a range of vascular tissues.

Rings of guinea-pig portal vein (GPPV), rabbit portal vein (RbPV) and rat aorta (RA; endothelium removed), were suspended in Krebs' under resting loads of 2g (RA) and 0.5g (GPPV, RbPV). A 2cm length of rabbit ear artery (RbEA) was perfused with Krebs' to give 15mmHg basal perfusion pressure. The GPPV and RbPV were pretreated with 0.1μM BQ3020 (Ihara et al., 1992b) to selectively desensitise any ET_B receptors. Partial concentration response curves were constructed to either ET-1 or ET-3 followed by 2h washout. BQ123 was equilibrated for 30 min then the agonist curve repeated. pA_2 values were calculated from the concentration ratios obtained with a single concentration of BQ123 (0.01-1μM).

Table 1

Tissue	Potency ratio of ET-1 : ET-3 (95% conf. limits)	pA_2 for BQ123 vs ET-1	± s.e.m vs ET-3
RA	13 (8-22)	7.2±0.1	8.4±0.1
GPPV	34 (19-58)	7.4±0.1	8.4±0.2
RbPV	58 (20-174)	7.2±0.2	8.7±0.0
RbEA	15 (5-71)	6.5±0.2	8.3±0.1

In all tissues ET-1 was more potent than ET-3 although the potency ratio varied. The pA_2 value for BQ123 against ET-3 was consistent across tissues and always significantly ($p<0.01$) higher than against ET-1. There was also greater variability of pA_2 values with ET-1 as agonist (Table 1).

These results agree with our previous findings in guinea-pig left atrium where pA_2 values for BQ123 against ET-3 were 8.5-8.7 but the antagonist was much less potent against ET-1 (Hatton et al., 1994). It is unlikely that ET_B receptors are involved in these responses. It is possible that different subtypes of the ET_A receptor may account for the different pA_2 values. Alternatively, it is hypothesised that ET-1 may access additional ET_A receptor sites that are less accessible to ET-3 and BQ123.

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551P INHIBITION OF ENDOGENOUS SYNTHESIS OF ENDOTHELIN-1 IN CULTURED HUMAN AND BOVINE ENDOTHELIAL AND HUMAN EPITHELIAL CELLS BY PHOSPHORAMIDON AND LEUPEPTIN

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Endothelin-1 (ET-1) is synthesised within endothelial cells from its precursor, big ET-1 by the putative endothelin converting enzyme (ECE). We have shown that ET-1 is contained within a subcellular fraction of bovine aortic endothelial cells (BAEC) suggesting that ET-1 is processed intracellularly within transport vesicles (Harrison *et al.*, 1993). Consistent with other groups we have shown that endogenous production of ET-1 in BAEC is inhibited by 100μM phosphoramidon (PHA) (Corder *et al.*, 1993). However in contrast to its effect on BAEC, 100μM PHA does not significantly reduce levels of ET-1 production in the human endothelial cell line, EA.hy 926 Hence, we have investigated the effect of higher concentrations of PHA on ET-1 biosynthesis by human endothelial and epithelial cells. A cysteine protease ECE has been identified and partially purified from porcine aortic endothelial cells (Deng *et al.*, 1993). Therefore we have investigated the effects of leupeptin, a reversible cysteine protease inhibitor, on the production of ET-1 from endothelial cells.

BAEC, human umbilical vein endothelial cells (HUVEC), EA.hy 926 and A549 (human lung carcinoma epithelial) cells were grown to confluence in 6 well plates in an atmosphere of 5%CO_2 in air at 37°C. BAEC and HUVEC were used up to passage 4. EA.hy 926 and A549 cells were studied between passage 35-80 and 10-20 respectively. All cells were treated for 24h in serum free conditioning medium at 37°C. 1μM PHA was included in control wells for EA.hy 926 and HUVEC to prevent ET-1 degradation by neutral endopeptidase 24.11. ET-1 was measured in each sample using a C-terminal specific RIA for $ET_{(16-21)}$ (Corder *et al.*, 1993). ET-1 release as % control is expressed as mean ± s.e.m (n = 4-9). Mean control release (fmol/well/24h) is indicated in Table 1.

Table 1. ET-1 (mean ± s.e.m) expressed as % control release

	Control	PHA 100μM	PHA 1mM	Leupeptin 100μM	Leupeptin 1mM
BAEC	2590 ± 302	22.1 ± 1*	3.6 ± 0.1*	69.5 ± 9.9 *	33.5 ± 3.0 *
HUVEC	608 ± 70	66.9 ± 8.9*	7.4 ± 1.8*	84.0 ± 4.7 *	44.2 ± 2.8 *
EA.hy 926	307 ± 23	89.8 ± 7.3	11.1 ± 0.04*	63.5 ± 5.5 *	28.5 ± 2.7 *
A549 (epithelial)	392 ± 40	67.0 ± 4.6*	24.0 ± 2.8*	N/D	N/D

(*$p<0.05$ versus control, analysis by two-tailed students t-test. N/D = not determined)

These results demonstrate that human cells require higher concentrations of PHA for effective inhibition of the intracellular ECE involved in ET-1 biosynthesis. In comparison leupeptin was equally effective at suppressing ET-1 levels on bovine and human cells However the mechanism of this effect and the enzyme involved awaits to be determined.

Supported By Parke-Davis Pharmaceutical Research Division of Warner-Lambert and The Wellcome Trust

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552P POTENCY OF THE NOVEL ORALLY ACTIVE ENDOTHELIN ANTAGONIST Ro46-2005 FOR ENDOTHELIN RECEPTORS IN HUMAN VASCULAR SMOOTH MUSCLE

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The efficacy of the first synthetic orally active endothelin (ET) antagonist Ro46-2005 has been demonstrated in two animal models of pathophysiological vasospasm (Clozel et al., 1993). We have determined the potency of this proposed non-selective endothelin antagonist for ET receptors in human vascular smooth muscle preparations.

Saphenous vein (SV) and internal mammary artery (IMA) were obtained from patients receiving coronary artery bypass grafts; coronary artery (CA) from cardiac transplant recipients. To identify ET receptor subtypes in media of SV reverse transcriptase-polymerase chain reaction (RT-PCR) using nested oligonucleotide primers was carried out as described (Davenport et al., 1993). For competition binding assays SV membranes were incubated for 2 hours at 22°C with 0.1nM [^{125}I]ET-1and increasing concentrations (20pM-100μM) of Ro46-2005 or the ET_A antagonist BQ123 (Davenport et al., 1993). Total [^{125}I]ET-1 binding was determined in the absence of antagonists and non-specific binding by inclusion of 1μM ET-1. Values for affinity (K_D) and binding density (B_{max}) were determined using the iterative curve fitting programme LIGAND. For *in vitro* pharmacological studies 2mm rings of endothelium-denuded CA, SV and IMA (n=6) were suspended under 2, 1 and 2g resting tension respectively in organ baths containing Krebs-Henseleit solution (37°C). Cumulative dose-response curves (DRC's) were constructed to ET-1 (10^{-10}-$3x10^{-7}$M) in the absence (control) and presence of Ro46-2005 (3-30μM) added to the bathing medium 30 minutes earlier. ET-1 responses were expressed as a % of that to 50mM KCl. pA_2 Values were obtained by analysis of the Schild regressions.

RT-PCR detected mRNA encoding ET_A and ET_B receptors in media of SV (n=3). BQ123 competed for specifically bound [^{125}I]ET-1 in SV in a biphasic manner with ET_AK_D of 0.55±0.17nM and ET_BK_D of 14.4±2.6μM (pooled data mean ± s.e.mean n=3: ET_A:ET_B 80:20%). Ro46-2005 competed with a K_D of 0.15±0.01μM (n=3) with a one-site fit preferred to a two or three site model. This is consistent with Ro46-2005 being non-selective for human ET_A and ET_B receptors. In functional studies Ro46-2005 had no agonist activity but caused parallel rightward shifts of the ET-1 DRC without attenuation of the maximum response. Schild-derived pA_2 values of 5.6 in CA, 6.2 in SV and 5.8 in IMA were obtained. Slopes of the regression lines were close to 1.

These values are similar to that obtained for antagonism of ET_A-mediated contraction of rat aorta (Clozel et al.,1993) and support our observations that in those human vessels investigated vasoconstriction occurs principally via the ET_A receptor (Maguire and Davenport, 1993; Davenport and Maguire, 1994).

We are grateful to the Theatre staff at Papworth Hospital for permission to collect tissue samples.

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553P EFFECTS OF ENDOTHELIN ANTAGONISTS ON VASOCONSTRICTOR AND VASODILATOR RESPONSES TO THE ET_B AGONIST BQ3020 IN PITHED RATS

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It has been proposed that the endothelin ET_B receptor mediates both vasoconstriction and vasodilatation in the rat (Clozel et al., 1992). We have tested this hypothesis by studying the haemodynamic effects of a selective ET_B agonist BQ3020 ([Ala^{11,15}]Ac-ET-$1_{(6-21)}$, (Ihara et al., 1992a)) in pithed rats using the mixed ET antagonist Ro46-2005 (Clozel et al., 1993) and the selective ET_A antagonist BQ123 (Ihara et al., 1992b). Alderley Park rats (320-380g) were anaesthetised with halothane and artificially respired through a tracheal cannula with 40% O_2, 60% N_2. Rats were pithed and the right carotid artery and left femoral vein were cannulated for measurement of mean arterial pressure (MAP) and administration of drugs respectively. Mesenteric blood flow was assessed using a pulsed Doppler flow probe positioned around the superior mesenteric artery. BQ3020 (0.1-3.0 nmolkg^{-1}) was administered cumulatively and changes in MAP and mesenteric conductance (MC) were calculated. Results are shown for the 1.0 nmolkg^{-1} dose. In some animals either BQ123 (0.3 mgkg^{-1}) or Ro46-2005 (30 mgkg^{-1}) was administered 5 minutes prior to commencing addition of BQ3020.

Treatment	Control (n=6)	BQ123 (n=5)	Ro46-2005 (n=4)
Control MAP (mmHg)	59 + 1	59 ± 1	55 ± 0
Initial Δ MAP (% control MAP)	-18.6 + 3.5*	-18.7 ± 2.0 *	14.1 ± 1.1*†
Delayed Δ MAP (% control MAP)	19.1 + 7.2*	14.3 ± 5.0	12.3 ± 1.4*
% change in MC	-33.2 ± 8.7*	-38.7 ± 6.4*	-22.5 ± 2.7*

* $p< 0.05$, paired Students t-test vs pretreatment control; † $p < 0.05$ unpaired Students t-test vs control group.

BQ3020 induced a biphasic MAP response consisting of a dose-dependent initial (within 30 sec) depressor response followed by a small pressor response at doses greater than 0.3 nmolkg^{-1}. BQ3020 also produced a prolonged mesenteric constriction at these doses. Administration of neither BQ123 nor Ro46-2005 was associated with haemodynamic effects. BQ123, at a dose which in seperate experiments inhibited the pressor response to 1.0 nmolkg^{-1} big ET-1 in pithed rats by 76 ± 4% (n=6), did not inhibit the MAP or MC responses to BQ3020, suggesting that ET_A receptors are not involved. Ro46-2005, at a dose which inhibited pressor responses to 1.0 nmolkg^{-1} big ET-1 in pithed rats by 56 ± 5% (n=4)), inhibited the initial depressor response to BQ3020 (1.0 nmolkg^{-1}), but had no effect on the decreased mesenteric constriction or delayed pressor response. It is possible that differences in ET_B receptor subtype may account for this difference in sensitivity to Ro46-2005.

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554P [^{125}I]PD151242 IS SELECTIVE FOR ENDOTHELIN ET$_A$ RECEPTORS IN HUMAN KIDNEY AND LOCALISES TO THE VASCULATURE

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The linear tetrapeptide radioligand, [^{125}I]-PD151242 has been shown to bind with high, nanomolar affinity and selectivity to ET$_A$ receptors in the human vasculature which contains predominantly the ET$_A$ sub-type. The unlabelled compound was a competitive antagonist, causing a parallel rightward shift of the endothelin-1 (ET-1) concentration response curve in isolated epicardial coronary arteries (Davenport *et al.*, 1994). We have previously shown that the human kidney contains more than 70% ET$_B$ receptors (Karet *et al.*, 1993) and our aim was to characterise [^{125}I]-PD151242 in this ET$_B$ rich tissue.

Saturation experiments were carried out using 10 μm thick cryostat sections of human kidney. Sections were incubated with increasing concentrations (8 pM-8 nM) of [^{125}I]-PD151242, N-[(-hexahydro-1-azepinyl)carbonyl]L-Leu(1-Me)D-Trp-D-Tyr, (Amersham International, IM272) in incubation buffer for 2 h at 23°C (Davenport *et al.*, 1993). Sections were rinsed in Tris-HCl buffer (0.05M, pH 7.4) at 4°C (3x5min) and the amount of radioactivity measured in a gamma counter. In competition assays, sections were incubated with 100 pM [^{125}I] PD151242 and increasing concentrations of unlabelled peptides (20pM-100μM). Non-specific binding was defined using 1μM of the appropriate unlabelled compound.

In the kidney, saturation binding assays using [^{125}I]-PD151242 revealed a single population of high affinity ET receptors: $K_D = 0.75 \pm 0.07$ nM and $B_{max} = 48.4 \pm 1.56$, fmol/mg protein (n = 3 individuals ± s.e.mean). Hill slopes were close to unity and a one site fit was preferred to a two site model. ET$_A$ selective ligands competed for [^{125}I]-PD151242 binding with sub-nanomolar affinity: BQ123 (Nakamichi *et al.*, 1992) $K_D = 0.43 \pm 0.10$ nM; FR139317, (Aramori *et al.*, 1993) $K_D = 0.37 \pm 0.06$ nM. In each case, a one site fit was preferred to a two site model. In contrast, the ET$_B$ selective BQ3020 (Molenaar *et al.*, 1993) only competed for the binding of [^{125}I]-PD151242 in the micromolar range. Autoradiographical studies showed that [^{125}I]-PD151242 was mainly localised to the small vessels particularly in the cortex, whereas [^{125}I]-BQ3020 binding was more heterogeneous and concentrated in the medulla. The results suggest that [^{125}I]-PD151242 is selective for ET$_A$ receptors in human kidney and this sub-type is mainly localised to the renal vasculature.

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555P THE EFFECT OF COOLING ON THE RESPONSE TO ENDOTHELIN-1 IN ISOLATED RAT MESENTERIC RESISTANCE ARTERIES

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Clinical studies (Zamora *et al.*, 1990) implicate endothelin-1 (ET-1) in mediating the prolonged cold-induced vasospasm of Raynaud's Disease (RD), the pathogenesis of which is unknown. Using cutaneous rabbit ear arteries, Monge *et al.* (1991) have shown cooling decreases sensitivity to ET-1, perhaps by increasing availability of nitric oxide (NO). If the endothelium in RD lacks this NO-mediated inhibitory function during cooling, increased production and enhanced constriction to ET-1 might lead to vasospasm. The present study used a rapid cooling device to examine the effect of temperature on the response to ET-1 in non-cutaneous resistance arteries, since an association between RD and other vasospastic conditions, such as migraine and variant angina, suggests that a common vascular defect exists.

Mesenteric vascular beds were removed from male Wistar rats (11-16 weeks) and placed in a dissecting dish containing cold physiological salt solution. Second and third order resistance arteries with a mean diameter of 279±8µm (n=24) were excised and cannulated in a small vessel arteriograph (Living Systems Instrumentation, USA) at 37°C. A pressure servo unit maintained intraluminal pressure, without flow, at 60mmHg, and the lumen diameter was measured using a video dimension analyser. Following 60-90 min equilibration, three doses of phenylephrine (PE, 10^{-5}M) were given to produce a contraction to < 35% of resting lumen diameter. Acetylcholine (ACh, 10^{-6}M) was given during the third contraction to assess endothelial integrity. Removal of endothelium was achieved by passing air through the lumen of the vessel (Falloon *et al.*, 1993) and confirmed by the loss of ACh (up to 10^{-4}M)-induced relaxation during constriction to a fourth dose of PE. Cooling was achieved by passing the superfusate through a rapid heat-exchange element (Moor Instruments, U.K.) before it entered the arteriograph. Four sets of experiments (n=6 per set) were carried out: at 37°C or 24°C, and with endothelium either intact or removed. Cumulative concentration-response curves to ET-1 (10^{-12} to 3×10^{-7}M) were obtained for each set. Responses are expressed as a percentage of the maximum contraction to PE. EC_{50} and E_{max} values are shown as mean ± s.e.mean.

ET-1 produced a concentration-dependent constriction in all vessels. Removal of the endothelium and/or cooling did not cause a significant change in resting lumen diameter. At 37°C, arteries with endothelium removed had a more than 2-fold greater sensitivity to ET-1 than arteries with intact endothelium (EC_{50} = 1.0±0.2 vs 2.4±0.4 x 10^{-9}M, $P<0.02$; unpaired t-test). Similarly, at 24°C, removal of the endothelium resulted in a 2-fold increase in sensitivity to ET-1 compared to the intact vessel (EC_{50} = 6.3±1.3 x 10^{-10}M vs 1.4±0.2 x 10^{-9}M, $P<0.01$). There was a significant increase in the E_{max} for ET-1 after de-endothelialisation, at both 37°C (from 101±2 to 108±1, $P<0.01$) and 24°C (from 101±2 to 108±1, $P<0.02$). When the effect of temperature on arteries with endothelium was examined, it was seen that cooling to 24°C caused a 1.7-fold increase in sensitivity to ET-1 ($P<0.05$). After denudation, this leftward shift of the concentration-response curve to ET-1 was still apparent, but this was only significant at higher concentrations (10^{-9} and 3 x 10^{-9}M, $P<0.05$ and $P<0.001$), and not for EC_{50}.

These results show that endothelium-derived dilator substances oppose vasoconstriction to ET-1 in rat mesenteric resistance arteries at both 37 and 24°C. Cooling increases sensitivity to ET-1 in vessels with endothelium present. When the endothelium is absent, increased sensitivity only occurs at higher concentrations, suggesting that endothelium-dependent effects are more important at lower concentrations, whilst at higher concentrations there is enhanced sensitivity of vascular smooth muscle. These studies suggest that endothelin may be implicated in cooling-induced vasoconstriction, though the role of endothelium-derived factors in these vessels requires further investigation.

This work was supported by the British Heart Foundation. PJWS is the recipient of an AJ Clark Studentship from the British Pharmacological Society.

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556P THE POSSIBLE INVOLVEMENT OF NITRIC OXIDE IN DESENSITISATION TO 5-HYDROXYTRYPTAMINE IN RAT PULMONARY ARTERY RINGS

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5-Hydroxytryptamine (5-HT) has complex effects in blood vessels, in which pressor responses to 5-HT show a characteristic desensitisation that may be partly due to release of nitric oxide (NO) from the endothelium (Wakabayashi, 1993). The present study investigated this possibility by examining the effects of 5-HT in pulmonary artery rings in the absence and presence of the endothelium and of the nitric oxide synthase inhibitor, N-nitro-L-arginine methyl ester (L-NAME), which potentiates the effects of 5-HT in the saline-perfused pulmonary vasculature (Shaw et al, 1990). Male Wistar rats (200-250g) were killed by stunning and bleeding. Rings (3-4mm) of main and branch pulmonary arteries were dissected, mounted under 1g tension on stainless steel hooks in 25ml organ baths containing Krebs-Henseleit solution (37°C), gassed with 95% O_2/5% CO_2). In some experiments the endothelium was removed by rubbing the luminal surface of the rings with a wooden rod. The effectiveness of this procedure was monitored using acetylcholine (ACh, 1μM), which normally caused relaxation but had no effect in rubbed rings. Tension responses (mg mg^{-1} tissue were recorded isometrically and concentration-response curves (CRC) to 5-HT (10^{-7}-10^{-3}M) and phenylephrine (PE, 10^{-9}-10^{-3}M)) were obtained in the absence and presence of L-NAME (500 μM). In some experiments, the rings were exposed to 5-HT (10^{-4}M, 30 min), which caused desensitisation. Standard responses and CRC to 5-HT (10^{-7}-10^{-3}M) and to phenylephrine (PE, 10^{-9}-10^{-4}M) were obtained and compared with controls obtained prior to desensitisation.

Prolonged exposure of main and branch pulmonary artery rings to 5-HT produced marked desensitisation of all responses to 5-HT including the maximum but only reduced responses to low concentrations (1-10nM) of PE. This desensitisation was most marked in intact main pulmonary artery rings. Removal of the endothelium increased responses to 5-HT and reduced but did not abolish the desensitisation. The standard response (mg mg^{-1} tissue) to 5-HT (100 μM), given before tachyphylaxis, was 156±11 (mean ± s.e. mean, n=6) in intact rings and 220±8 in endothelial denuded rings (0.01>P>0.001). After tachyphylaxis this response was reduced to 16±1 in intact rings and to 96±16 in denuded rings (0.05>P>0.01). Prior treatment with L-NAME (500 μM) had a similar effect. In intact rings, in the presence of L-NAME and before tachyphylaxis, the standard response was 224±34 and in denuded rings was 204±18. After tachyphylaxis in the presence of L-NAME, this response was reduced to 92±17 (0.05>P>0.01) in intact rings and to 120±12 (0.01>P>0.001) in denuded rings. The addition of L-NAME (500 μM) to the desensitised rings during prolonged exposure to 5-HT (100 μM), produced a contraction that was reversed by L-arginine (200 μM). These results suggest that release of basal endothelial NO contributes to but does not completely explain the desensitisation caused by 5-HT in the rat pulmonary artery.

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557P THE CARDIOVASCULAR EFFECTS OF 5-HT $_{1A}$ SELECTIVE COMPOUNDS: ANTAGONIST STUDIES WITH WAY-100,135

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* 8-OH-DPAT causes a centrally mediated fall in blood pressure and heart rate in the anaesthetised and conscious rat via stimulation of central $5HT_{1A}$ receptors (Fozard *et al*, 1990 ; Connor & Higgins, 1990); an effect blocked by the $5HT_{1A}$ antagonist UH-301 (Bjork *et al*, 1991). This study seeks to characterise further the action of $5HT_{1A}$ agonists and partial agonists on heart rate in the anaesthetised rat, in particular their interaction with the novel $5HT_{1A}$ antagonist WAY-100,135 (Fletcher *et al*,1993). Male rats (370-400g) were anaesthetised with pentobarbitone sodium, 60 mg/kg i.p. The carotid artery and jugular vein were cannulated for blood pressure measurement and compound administration respectively; heart rate was measured via subcutaneous ECG electrodes. $5HT_{1A}$ agonists were administered cumulatively at ten minute intervals to allow a single dose-response curve to be constructed and results were expressed as a percentage fall from initial resting heart rate. In antagonist studies, WAY-100,135 (1mg/kg i.v.) was administered ten minutes prior to the agonist challenge. 8-OH-DPAT produced a maximum fall in heart rate of 35.4±1.2% (n=20), an ED_{20} (the dose to cause 20% fall in heart rate) was used as a measure of potency in subsequent experiments. * the (R) form of 8-OH-DPAT was used in this study.

COMPOUND	8-OH-DPAT	8-OH-DPAT+ WAY-100,135	BUSPIRONE	BUSPIRONE +WAY-100,135	BMY-7378	BMY-7378 +WAY-100,135	FLESINOXAN	FLESINOXAN +WAY-100,135
n	27	24	6	8	4	4	5	5
PRE-AGONIST HR	396 ± 6	358 ± 9	391 ±15	361 ± 13	388 ± 24	388 ± 9	401 ± 15	351 ± 12
MAX FALL HR (%) ± SE	35 ± 1	24 ± 2	22.0 ± 1	23 ± 3	20.0 ± 1	19 ± 4	36 ± 4	25 ± 1
ED_{20}mg/kg	0.014	0.18	0.66	1.8	0.1	>3.0	0.12	0.54

In conclusion, compounds with agonist activity at $5HT_{1A}$ receptors produce dose-related falls in heart rate in the anaesthetised rat. 8-OH-DPAT and flesinoxan produced greater maximum falls than buspirone and BMY-7378, consistent with the latter having partial agonist activity. All these responses were attenuated by pretreatment with WAY-100,135, confirming that these effects are mediated via stimulation of $5HT_{1A}$ receptors. It should be noted that WAY-100,135 itself caused a modest fall in heart rate of 7.4% (30 bpm, n=41) which contrasts with the lack of effect seen in other studies with UH 301 (Bjork *et al*, 1991); the mechanism underlying these falls require further investigation. Therefore this model, used in conjunction with WAY-100,135, can be used to determine if compounds possess central $5HT_{1A}$ agonist activity *in vivo*.

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558P HETEROGENEITY OF 5-HT RECEPTORS IN HUMAN SIGMOID COLON

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5-HT has previously been shown to inhibit the spontaneous contractions of human colonic circular muscle via a 5-HT_4 receptor (Tam *et al.*, 1992). In the present study, we have examined the effects of 5-HT on the basal tone of this tissue. We would also like to present preliminary data on the secretory effect of 5-HT in human sigmoid colonic mucosa. After storage overnight at 4°C, strips of circular muscle were set up under isotonic conditions and a preload of 1g in oxygenated Krebs at 37°C. A single cumulative response curve to 5-HT was obtained, either in the absence or presence of antagonist. Results were expressed as % of maximum relaxation to isoprenaline (80μM). For secretory studies, sigmoid colonic mucosa was stripped of muscle layers and voltage-clamped in Ussing chambers where changes in short-circuit current were monitored (Burleigh, 1991). Because the response to 5-HT showed rapid desensitisation, only a single concentration of 5-HT was applied to any one tissue, either in the absence or presence of antagonist. Results were expressed as % of maximum response to forskolin (25μM). In both studies, antagonists were incubated for 30 min before adding the agonist. Data were analyzed using the Mann-Whitney U test, with $p<0.05$ taken to indicate significance. EC_{50} values are geometric mean with 95% confidence limits (95% C.L.).

In circular muscle, 5-HT caused a concentration-dependant relaxation, with an EC_{50} of 0.24μM (0.16-0.34, n=15). As shown in table 1, only DAU 6285 (a selective 5-HT_4 receptor antagonist; Schiavone *et al.*, 1992) caused a significant increase in EC_{50} of 5-HT, leading to a single-dose pA_2 estimate of 6.81±0.23 (between paired tissues, as described by Mackay, 1978).
Application of 5-HT to the serosal side of sigmoid colonic mucosa led to a concentration-dependant increase in short-circuit current. The effect of antagonists was tested against a single dose of 10μM 5-HT (approximate EC_{75}). Ketanserin (1μM) abolished the secretory response to 10μM 5-HT (n=4), and at a concentration of 10nM led to a significant reduction in the 5-HT response (88.2±4.9% reduction, n=10, $p<0.05$). Ondansetron (10μM) and DAU 6285 (10μM) had no significant effect on the response to 10μM 5-HT, when applied separately (n=4, $p>0.05$) or together (n=6, $p>0.05$).
Application of 10μM 5-carboxyamidotryptamine (a selective agonist at 5-HT_1 receptors) to the tissue produced no change in basal short-circuit current (n=3, $p>0.05$).
In conclusion, the relaxant effect of 5-HT in human sigmoid colonic circular muscle is mediated by a receptor of the 5-HT_4 sub-type, whereas the electrogenic secretory effect in mucosa appears to be mediated by a 5-HT_2 receptor.

This study was supported by The Sir Jules Thorn Trust.

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Treatment	EC_{50} (μM)[a] [95% C.L.]	n
Control	0.24 [0.16-0.34]	15
Methysergide (10μM)	0.15 [0.09-0.23]	10
Ketanserin (1μM)	0.16 [0.08-0.32]	10
Ondansetron (10μM)	0.17 [0.07-0.42]	10
DAU 6285 (10μM)	13.42* [4.70-38.38]	10

Table 1. EC_{50} value of 5-HT [(a)] causing relaxation of human sigmoid colonic circular muscle in absence (control) or presence of antagonists.
* indicates $p<0.05$ compared to control.

559P 5-HT_4 RECEPTOR-MEDIATED CONTRACTION OF COLON IN THE ANAESTHETIZED GUINEA-PIG

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5-HT_4 receptor agonism causes smooth muscle contraction of the proximal and distal colon of guinea-pig *in vitro* (Elswood et al., 1991; Wardle & Sanger, 1993). However, the role of 5-HT_4 receptors in contraction of colonic smooth muscle *in vivo* has seldom been investigated and was the focus of this study.

Male Hartley guinea-pigs (Harlan, 350-450g) were anaesthetized with inactin (80 mg·kg, i.p.) and artificially ventilated with a Harvard respirator. The abdominal viscera were exposed and a strain gauge was sutured onto the ascending colon in order to measure circular muscle tension. Following a 1h stabilization period, each animal was pre-treated with methysergide (3 $mg \cdot kg^{-1}$, iv) and granisetron (1 $mg \cdot kg^{-1}$, iv) in order to block 5-HT_1, 5-HT_2 and 5-HT_3 receptors. Fifteen minutes later, a non-cumulative dose-effect curve to the appropriate agonist was constructed. The contractile response to each agonist was expressed as a percent of the response to a maximal dose of carbachol (100 $\mu g \cdot kg^{-1}$, iv) in the same preparation. In a separate series of experiments, each animal was treated with either saline or SB 204070 (0.1 $mg \cdot kg^{-1}$, iv), in addition to methysergide and granisetron, prior to the construction of a dose-effect curve to 5-hydroxytryptamine (5-HT).

5-HT, 5-methoxytryptamine (5-MeOT), SC 53116 (Flynn et al., 1992) and 2-methyl 5-hydroxytryptamine (2-me-5-HT) produced dose-dependent increases in circular muscle tension with the following rank order of potency [ED_{50}; $\mu g \cdot kg^{-1}$, iv,(95% confidence limits)]: 5-HT [1.5 (0.9 - 2.2)] > SC 53116 [9.2 (5.2 - 16.1)] > 5-MeOT [55.1 (27.4 -110.9)] >>> 2-me-5-HT [> 316].

SB 204070 (0.1 $mg \cdot kg^{-1}$, iv), a selective 5-HT_4 antagonist (Wardle et al, 1993), produced a dextral and unsurmountable shift of the dose-effect curve to 5-HT. The ED_{50} ($\mu g \cdot kg^{-1}$, iv) and maximal response (% of carbachol maximum) to 5-HT was 3.0 (1.4 -6.4) and 109.6 (77.8 - 141.5), respectively, in saline-treated rats (n = 6) whereas it was 10.5 (7.1 - 15.5) and 44.4 (34.6 - 54.1), respectively, in SB 204070-treated rats (n = 7).

These data suggest that, under the operative experimental conditions, agonism of 5-HT_4 receptors can cause contraction of colonic circular muscle in the anaesthetized guinea-pig. This animal model appears to offer a convenient method for assessing the gastrointestinal effects of 5-HT_4 agonists and antagonists *in vivo*.

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560P EVIDENCE OF 5-HT$_4$ RECEPTOR INVOLVEMENT IN THE FACILITATION OF THE PERISTALTIC REFLEX IN THE MARMOSET ILEUM

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5-HT$_4$ receptor activation has been shown to facilitate the peristaltic reflex in the guinea-pig isolated ileum (Costall *et al.*, 1993). In the present study, we investigated the peristaltic reflex in the marmoset isolated ileum and present evidence for a 5-HT$_4$ receptor involvement in the facilitation of the peristaltic reflex.

Anaesthetised marmosets (*Callithrix jacchus*, University of Bradford bred) of either sex were killed by decapitation and segments of ileum about 5-6 cm in length and from a distance of 1-15 cm from the ileo-caecal junction were cannulated at the oral and aboral ends and secured horizontally in perspex baths containing Krebs-Henseleit solution at 37°C and oxygenated with 95%O_2 and 5%CO_2. The oral ends were connected to peristaltic pumps from which oxygenated Krebs-Henseleit solution was introduced at a constant rate of 0.5 ml min^{-1}. The aboral ends were connected to the tubes which had two outlets; one at the level and the other 10 cm above the level of the fluid in the bath. The upper outlet was a bent narrow vertical tube of 3mm internal diameter. Any volume expelled from this outlet was collected in a 5 ml syringe connected to a pressure transducer so that recording of the volume expelled could also be made. The intraluminal pressure was measured from the aboral side. The tissues were equilibrated for 3-4 hour. The lower outlet was left open during the equilibration and the resting periods between the doses. The agonists were added to the serosal side either 1-3 min after the fluid started to overflow from the upper outlet, or at the end of the peristaltic stroke in tissues exhibiting continuous peristalsis after closure of the lower outlet. The concentration-response curves to agonists were constructed in a non-cumulative manner with an initial "priming" of the tissues with 0.1 μM 5-hydroxytryptamine (5-HT) and with at least a 15 min resting period between each concentration. Each tissue was used to construct only one concentration-response curve. A facilitatory response was defined as any decrease in the threshold for peristalsis below the threshold in the peristaltic stroke just before the addition of drug (in tissues undergoing peristalsis) or below the pressure attained when the fluid was overflowing from the top outlet (10cm of water) and was expressed as the percentage of the latter value. GR 113808 was equilibrated for at least 1 hour and was continuously present in the bath during the construction of the concentration-response curves to 5-HT or 5-methoxytryptamine (5-MeOT).

5-HT (0.01-10μM), 5-MeOT (0.01-10μM), 5-carboxamidotryptamine (0.1-10μM) and 2-methyl-5-HT (1-10μM) caused concentration-dependent reductions in the threshold for the peristaltic contractions with pEC_{50} (mean±s.e.mean) values of 7.85±0.19 (n=6), 7.79±0.24 (n=4), 6.66±0.13 (n=3) and 6.24±0.16 (n=2) respectively. In the presence of GR 113808 (30nM) the concentration-response curves to both 5-HT and 5-MeOT were dextrally shifted with no reduction in the maximum response. The pEC_{50} values of 5-HT and 5-MeOT in the presence of GR 113808 were significantly decreased (Student t-test unpaired one tail) to 6.98±0.24 (n=3) and 6.83±0.36 (n=3) respectively.

The rank order of potency for the indole agonists to facilitate the peristaltic reflex and the ability of the selective 5-HT$_4$ receptor antagonist GR 113808 (Grossman *et al.*, 1993) to antagonise the 5-HT and 5-MeOT response indicates the presence of a 5-HT$_4$ receptor which can influence the peristaltic reflex in the marmoset ileum.

We thank the ORS Awards Scheme for providing an ORS award to B. R. Tuladhar and Glaxo for the gift of GR 113808.

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561P THE EFFECTS OF 5-HT ON TOTAL cGMP CONTENT AND PERFUSION PRESSURE OF THE ISOLATED RAT LUNG

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In the isolated pulmonary vasculature of the rat, perfused with Krebs buffer, 5-HT has little or no effect until endogenous nitric oxide (NO) is inhibited (Shaw et al., 1992). The present study sought to determine if pressor responses to 5-HT in the pulmonary vasculature of the rat are attenuated by nitric oxide released spontaneously from the endothelium (basal release) or by 5-HT-induced release of NO from the endothelium, an event that has been reported in other arteries (Cocks and Angus, 1983). The pressor responses to 5-HT and total lung cGMP content, monitored as an index of endogenous NO, were measured in the absence and presence of L-N-nitro arginine methyl ester (L-NAME) an inhibitor of nitric oxide synthase. In addition, the ability of 5-HT, and for comparison carbachol, to induce relaxation of isolated preconstricted pulmonary arteries was examined.

Male Wistar rats (230 - 280 g.) were anaesthetised with pentobarbitone sodium (60 mg kg^{-1}, i.p.) and heparinised (2000 i.u. kg^{-1}, i.p.). The trachea was cannulated and the lungs respired artificially (stroke volume = 2.5 ml, rate = 55 min.$^{-1}$). The pulmonary artery and left atrium were cannulated and Krebs buffer (22°C) allowed to run through the pulmonary vasculature to remove blood cells. The pulmonary vasculature was then perfused at 12 ml per minute for 1 hour with Krebs buffer (35°C, gassed with 95%O_2/5%CO_2). Pulmonary artery pressure was monitored using a Statham pressure transducer. Bovine serum albumin 4% was added to the perfusate and the lungs perfused for a further 10 min. 5-HT (50μM) was added to the perfusate and perfused until an equilibrium response was achieved. After 90 sec. the lungs were freed from the connecting cannulae and rapidly frozen in liquid nitrogen. The lungs were partially thawed, homogenised and cyclic nucleotides extracted in 75% ETOH/H_2O (v/v). Extracts were dried under air, reconstituted in an acetate buffer, and the cGMP content measured by radioimmunoassay.

Basal cGMP content (in the presence of 2 mM IBMX) was 415 ± 67 fmol. mg^{-1} (total lung protein, n = 8). 5-HT (50μM) produced a small transient increase in perfusion pressure and increased the cGMP content to 963 ±184 fmol. mg^{-1} (n = 4 $p < 0.05$). In the presence of L-NAME (500 μM) the cGMP content was reduced to 114 ± 47 fmol mg^{-1} and 5-HT increased the perfusion pressure from 15.23 ± 4.1 mmHg to 39 ± 4 mmHg (n = 8, $p < 0.05$). In isolated pulmonary arteries carbachol, 5-HT and the 5-HT1 receptor agonists 5-carboxyamidotryptamine (5-CT) and sumatriptan relaxed preconstricted arteries. The relaxation induced by carbachol but not 5-HT, 5-CT or sumatriptan was abolished by L-NAME (500μM). The present study shows that 5-HT increases the cGMP content of the lung by a NO-dependent mechanism and that endogenous NO attenuates the 5-HT-induced pressor response. 5-HT relaxes preconstricted pulmonary arteries by a NO-independent mechanism suggesting that the L-NAME-sensitive increase in cGMP associated the 5-HT may not be mediated by a direct effect of 5-HT on the endothelium.

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562P REGIONAL HAEMODYNAMIC EFFECTS OF ADRENOMEDULLIN IN CONSCIOUS RATS

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Recently, the novel peptide, adrenomedullin (ADM) was shown to cause marked hypotension, but no tachycardia, in anaesthetised rats (Ishiyama *et al.*, 1993). Since cardiac and stroke index increased, the hypotensive effect of ADM was due to peripheral vasodilatation, but which vascular beds were involved is unknown. Therefore, we investigated the regional haemodynamic effects of ADM in conscious Long Evans rats chronically instrumented with pulsed Doppler flow probes and intravascular catheters; all surgery was carried out under sodium methohexitone anaesthesia (40-60 mg kg^{-1} i.p.). Animals received i.v. bolus injections of human ADM (Peptide Institute) at 0.3, 1, and 3 nmol kg^{-1} at intervals of at least 1 h, and responses were assessed from the areas under or over curves (AUC, $AOC_{0\text{-}60\ min}$, respectively). The results are summarised in Table 1.

Table 1. Integrated cardiovascular changes in response to ADM in the same, conscious Long Evans rats (n = 8). Values are mean ± s.e. mean; * $P < 0.05$ versus baseline; † $P < 0.05$ versus response to 0.3 nmol kg^{-1} ADM; ° $P < 0.05$ versus response to 1.0 nmol kg^{-1} ADM (Friedman's test).

Dose	0.3	1.0	3
Δ Heart rate (AUC; beats)	793 ± 299*	2073 ± 284*†	2604 ± 362*†
Δ Blood pressure (AOC; mm Hg min)	-104 ± 36*	-356 ± 76*†	-831 ± 68*†°
Δ Renal flow (AUC; kHz min)	33 ± 11*	39 ± 7*	56 ±14*
Δ Mesenteric flow (AUC; kHz min)	21 ± 8*	52 ± 11*	64 ± 16*
Δ Hindquarters flow (AUC; kHz min)	18 ± 4*	27 ± 7*	38 ± 10*
Δ Renal conductance (AUC; [kHz mm Hg^{-1}]10^3min)	366 ± 104*	620 ± 112*†	1198 ± 208*†°
Δ Mesenteric conductance (AUC; [kHz mm Hg^{-1}]10^3min)	238 ± 80*	722 ± 146*†	1257 ± 229*†°
Δ Hindquarters conductance (AUC; [kHz mm Hg^{-1}]10^3min)	192 ± 44*	404 ± 90*	774 ± 128*†°

ADM caused dose-dependent hypotension and tachycardia, accompanied by increases in renal, mesenteric and hindquarters blood flows, and dose-dependent dilatation in all 3 vascular beds. These results indicate that the absence of a tachycardic response to human ADM in the experiments of Ishiyama *et al.*, (1993) was probably due to the use of anaesthetised rats. Although ADM shows a slight structural homology with CGRP (Kitamura *et al.*, 1993), it appears that the latter has a more marked hypotensive action, (Gardiner *et al.*, 1988) whereas ADM is a more effective mesenteric vasodilator.

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563P TACHYKININ-ACTIVATED BLADDER CONTRACTIONS IN THE ANAESTHETISED GUINEA-PIG

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Tachykinin NK_2 receptors have been demonstrated in rat and guinea pig urinary bladder preparations (Falconieri-Erspamer et al, 1973; Maggi et al, 1991) and autoradiographic studies have localised NK_2 receptor sites to both the bladder smooth muscle and mucosa (Burcher & Buck, 1986; Carstairs et al, 1993). Studies *in vivo* have demonstrated contractile effects of NK_2 agonists on the bladder in anaesthetised rats (Lecci et al, 1993). In the present study we have examined the potential contributions of cholinergic and purinergic transmission to the bladder contractile response induced by the selective NK_2 receptor agonist, [βala^8]$NKA_{(4\text{-}10)}$, and compared this with the response produced in airways in the anaesthetised guinea pig. Female Dunkin Hartley guinea pigs (350-450g) were anaesthetised with urethane (1.2g/kg i.p.), the carotid artery was cannulated for blood pressure recording and the left jugular vein cannulated for i.v. drug administration. After tying off the external urethra, a catheter was inserted via the dome into the bladder for intraluminal pressure recording. The guinea pigs were artificially ventilated and the tracheal insufflation pressure recorded.

Administration of the NK_2 receptor agonist [βala^8]$NKA_{(4\text{-}10)}$ (5μg/kg, i.v.) caused a contraction of the bladder and an increase in tracheal insufflation pressure. Dose-response studies showed the bladder contraction to be of an "all or nothing" nature, with the threshold dose being approximately 1 to 5μg/kg. In contrast, the tracheal response was dose dependent and displayed an ED_{50} of 0.73±0.18μg/kg (mean±s.e.m., n=5). The NK_2 receptor antagonist SR48968 (1 - 300 μg/kg) abolished the bladder contraction at doses of 30 μg/kg and above. SR48968 inhibited the increase in tracheal insufflation pressure in a dose dependent manner, with an ED_{50} of 85.8±17.0μg/kg (mean±s.e.m., n=6). The bladder response to NK_2 receptor activation occurred in two distinct phases: an initial fast phase and a slower tonic component. Injection of atropine (1 to 1000μg/kg, i.v.) 5 minutes prior to [βala^8]$NKA_{(4\text{-}10)}$ resulted in a dose-dependent inhibition of the bladder contraction, with a maximum reduction of 63.2±6.7% (mean±s.e.m., n=5) being achieved at the highest dose. Treatment with the desensitising purinergic agonist, [αβ-Me]ATP (1-300μg/kg), 3 minutes prior to the NK_2 agonist, resulted in dose-dependent inhibition of the bladder contraction with a maximum inhibition of 34.8±6.7% (mean±s.e.m., n=5). Combination of atropine (300μg/kg) and [αβ-Me]ATP (300μg/kg) completely abolished the agonist induced bladder contraction (n = 5). Neither atropine nor [αβ-Me]ATP modified the [βala^8]$NKA_{(4\text{-}10)}$ (5μg/kg) induced increase in tracheal insufflation pressure.

In conclusion, in the anaesthetised guinea pig [βala^8]$NKA_{(4\text{-}10)}$ produces contractions of the urinary bladder by an indirect mechanism involving ACh and ATP. These cholinergic and purinergic components can be selectively inhibited using atropine and [αβ-Me]ATP respectively. This contrasts with the direct contractile effect of this selective NK_2 agonist on the guinea pig airways. The location and mechanism of action of the NK_2 receptors mediating effects of [βala^8]$NKA_{(4\text{-}10)}$ on the bladder remain to be determined.

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564P DOES RELAXIN INHIBIT MYOMETRIAL MECHANICAL ACTIVITY VIA A cAMP-DEPENDENT PATHWAY IN RAT UTERUS?

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This study examined if relaxin increased cAMP-dependent protein kinase activity (PKA activity ratio; the amount of PKA in the active form as a fraction of the total amount of PKA) in longitudinal myometrium (LM) or in intact uterus. Also, the potency of relaxin against oxytocin (OT)-induced spasm was determined in these two preparations in tissue bath studies. Relaxin was compared with salbutamol, a β_2-adrenoceptor agonist which activates PKA, and with levcromakalim, a K-channel opener.

Strips of LM or intact uterus were mounted for tension recording. A single, cumulative concentration-effect curve was constructed to each relaxant against OT (0.2 nM)-induced spasm. To measure PKA activity ratios, strips of LM or intact uterus were mounted for tension recording and exposed to OT (0.2 nM), tissues were incubated with relaxant for 1 min, then frozen and powdered, the powder was homogenised and centrifuged, and the supernatant assayed for PKA activity based on the Gibco BRL PKA assay system and Murray *et al.* (1990). Mean activity ratios were compared using the Kruskal-Wallis ANOVA followed by the Mann-Whitney test (Table 1).

Table 1 PKA Activity Ratios (n = 5) * P 0.05 ** P<0.01 (drug-exposed vs control)

Conc.	Salbutamol - LM	Salbutamol - Intact	Relaxin - LM	Relaxin - Intact	Conc.	Lev - Intact
0	0.17 ± 0.02	0.12 ± 0.01	0.16 ± 0.03	0.11 ± 0.02	0	0.12 ± 0.01
30nM	0.22 ± 0.03	0.15 ± 0.01	0.15 ± 0.03	0.13 ± 0.004	1 µM	0.12 ± 0.01
300 nM	0.30 ± 0.06*	0.42 ± 0.05**	0.20 ± 0.04	0.18 ± 0.01*	10 µM	0.12 ± 0.01
3 µM	0.32 ± 0.03**	0.49 ± 0.04*	0.19 ± 0.02	0.20 ± 0.02*	100 µM	0.13 ± 0.01

Incubation with salbutamol (300 nM, 3 µM) raised PKA activity ratio in both tissue preparations. The maximum stimulation was larger in the intact uterus (4.1 ± 0.3 fold) than in the LM (1.9 ± 0.3 fold). This difference may correlate with the increased potency (2.6-fold; P<0.05) of salbutamol against the OT-induced spasm in intact uterus compared to the LM. Levcromakalim inhibited the OT-induced spasm in the intact uterus (pD_2 = 6.91 ± 0.08) and did not alter PKA activity. In the LM relaxin induced a maximum 58 ± 5% inhibition of the OT-induced spasm at 2.5 µM and did not stimulate PKA activity. Relaxin completely inhibited the OT-induced spasm in the intact uterus (pD_2 = 7.91 ± 0.16) and at high concentrations (30 nM, 3 µM) induced a small rise in PKA activity ratio (1.8 ± 0.2 fold).

Relaxin inhibited mechanical activity in the LM by up to 60% without increasing the PKA activity ratio, indicating that this effect can be cAMP-independent. The circular myometrium and/or endometrium must be present, in addition to the LM, for the full relaxant action of relaxin. Relaxin produced a small stimulation of PKA activity in intact uterus suggesting a minor cAMP-dependent action is perhaps involved in the release of a mediator from the circular myometrium/endometrium which then acts on the LM.

S. Hughes was supported by the M.R.C.

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565P THE EFFECT OF Hoe-140 ON THE RESPONSE OF THE ISOLATED MOUSE UTERUS TO BRADYKININ AND RELATED COMPOUNDS

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The purpose of this study was to characterize the receptors involved in the response of the isolated mouse uterus to bradykinin (BK), Lys-BK and des-Arg^9-BK using the B_2 receptor antagonist Hoe-140 (Wirth *et al.*, 1991).

To synchronise oestrus mature female BKW mice were pretreated with stilboestrol (s.c., 100µg 100g^{-1}) 18h prior to the study. Uterine horns were superfused (2ml min^{-1}) with oxygenated Krebs solution (Sangha and Senior, 1989). After equilibration of the tissue agonists were injected directly into the flow of the superfusate, as bolus doses. As the profile of spontaneous activity and sensitivity to agonists often changed throughout the course of the experiments, comparisons were made between preparations in a non paired manner. Because of the variations in myogenic activity results have been normalised to take into account the level of background activity and are expressed as ratios of test response (T) to background activity (B), these results can then be expressed as $ED_{1.5}$ (excitatory dose) values, i.e the dose required to evoke a response one and a half times that of a background, spontaneous contraction (see Senior *et al.*, 1991). Indomethacin (2.79µM) was included in the perfusate and had no effect on myogenic activity.

The responses to BK (0.0005-10nmol), Lys-BK (0.001-10nmol) and des-Arg^9-BK (0.005-10nmol) were all qualitatively similar in that they each evoked monophasic contractions (n=7 in all experiments). The dose response curves to BK and Lys-BK were bell shaped and as can be seen from the table below, the responses (max T/B) elicited by des-Arg^9-BK and Lys-BK, were weak when compared to BK. The presence of Hoe-140 (3x 10^{-7}M) in the perfusate had no effect upon myogenic activity. However, the B_2 antagonist abolished the response to BK and significantly attenuated the response to Lys-BK. The presence of Hoe-140 had little effect upon the response to des-Arg^9-BK, except at the higher doses (5 and 10nmol) when it caused some reduction in response.

KININ	In the absence of Hoe-140		In the presence of Hoe-140	
	Mean $ED_{1.5}$ (nmol)	Max T/B (s.e.mean)	Mean $ED_{1.5}$ (nmol)	Max T/B (s.e.mean)
BK	0.0005	17.7±7.3	no response	no response
Lys-BK	0.01	4.8±0.7	10.0	2.2±0.5
des-Arg^9-BK	0.28	1.8±0.3	0.5	1.3±0.4

The results of this study confirm that the mouse uterus contains B_2 receptors and are in agreement with our earlier findings (Scott *et al.*, 1994); it would also appear that des-Arg^9-BK exerts some effect via this receptor type.

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566P α_1-ADRENOCEPTOR SUBTYPE MEDIATING CONTRACTILE RESPONSES OF THE HUMAN VAS DEFERENS

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It has been proposed that the human prostate possesses a population of functional α_{1C}-adrenoceptors (Marshall et al., 1993), but it is not known whether this receptor is found throughout the urogenital system in man. The present study examines the responses of the human vas deferens to determine the α_1-adrenoceptor subtype mediating the contractile responses of this tissue.

Segments (1cm) of human vas deferens were set up under 1g tension in Krebs-bicarbonate solution at 37°C. Non-cumulative concentration-response curves to phenylephrine were obtained in the absence or presence of WB4101 (3-30nM), abanoquil (1-10nM) prazosin (3-30nM) and nifedipine (1μM). A 30 min antagonist equilibration period was employed (60 min for abanoquil). Responses were also examined following incubation with chloroethylclonidine (CEC, 100μM for 40 min, 60min washout). Experiments were performed in the presence of cocaine (10μM), corticosterone (10μM) and propranolol (1μM).
WB4101 and prazosin produced rightward shifts of concentration-response curves without affecting maximum responses. Mean pK_B (± SEM) values of 9.2 ± 0.1 (n=18) and 8.6 ± 0.1 (n=12) were obtained for WB4101 and prazosin respectively. Schild plots for both antagonists had slopes similar to unity. Abanoquil at 1nM failed to shift phenylephrine concentration-response curves, but significantly reduced maximum responses by 66% from 1.63 ± 0.24g to 0.56 ± 0.06g ($P<0.01$). Higher concentrations of abanoquil (3 and 10nM) did not reduce maximum responses further, but did shift concentration-response curves to the right, the 10nM concentration yielding an apparent pK_B of 9.6 ± 0.1.

Incubation of tissues with CEC (100μM) did not significantly affect responses to phenylephrine. EC_{50} values and maximum responses to phenylephrine following incubation with CEC [49.1(27.1-88.8)μM; 0.89 ± 0.26g, n=6] were not significantly different from values for control curves [28.5(11.8-68.7)μM, 1.19 ± 0.36g]. Responses were depressed in the presence of 1μM nifedipine however, the maximum response to phenylephrine being reduced from 0.90 ± 0.24g to 0.61 ± 0.16g ($P<0.05$). Phenylephrine EC_{50} values were not affected by nifedipine.

The high potency of WB4101 and abanoquil, and the failure of CEC to antagonize responses suggests that the predominant functional α_1-adrenoceptor of the human vas deferens has the characteristics of the pharmacologically defined α_{1A}-adrenoceptor subtype.

S.J.McKinney was supported by the Nuffield Foundation.
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567P NORADRENALINE, BUT NOT 5-HYDROXYTRYPTAMINE, STIMULATES ACCUMULATION OF INOSITOL (1,4,5)-TRISPHOSPHATE IN RABBIT BASILAR ARTERY

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Inositol (1,4,5)-trisphosphate (IP_3) is formed in vascular smooth muscle cells following phospholipase C-mediated hydrolysis of membrane bound phosphoinositides. IP_3 discharges intracellular Ca^{2+} stores and stimulates contraction in vascular smooth muscle and is thought to couple receptors for vasoconstrictor agents to intracellular Ca^{2+} release and contraction. In a previous study, 5-hydroxytryptamine (5-HT) was found not to stimulate phosphoinositide hydrolysis, as measured by accumulation of [^{3}H]inositol phosphates, in the rabbit basilar artery, despite the potent contractile action of 5-HT on smooth muscle cells in this vessel (Clark & Garland, 1991). The present study examined the ability of 5-HT and noradrenaline to stimulate IP_3 accumulation in the rabbit basilar artery using a more direct method involving a protein binding assay.

Segments of endothelium-denuded rabbit basilar artery were bathed in warmed (37°C), oxygenated Krebs solution and noradrenaline or 5-HT applied for periods ranging from 5 s to 5 min. The reaction was terminated and IP_3 extracted with trichloroacetic acid and samples washed with diethylether and neutralized with Na_2HCO_3. The ability of tissue extracts to alter [^{3}H]IP_3 binding in bovine adrenal cortex microsomes was used to determine the amount of IP_3 in each sample (Challiss *et al.*, 1988). Noradrenaline (10 μM) stimulated a rapid, transient accumulation of IP_3 in the rabbit basilar artery, which was significantly greater than corresponding basal levels after 5 s and peaked after 15 s at 13.46 ± 4.23 pmol/ mg protein (n = 6) or 176 % of the corresponding basal level. IP_3 accumulation then declined to basal levels after 60 s before increasing again after 5 min to 13.21 ± 4.86 pmol/ mg protein (n = 4), or 300 % of the corresponding basal level. 5-HT (100 μM) did not stimulate significant IP_3 accumulation in rabbit basilar artery during the 5 min after its addition (n = 6). In rabbit basilar arteries bathed in Krebs solution containing 20 mM added K^+ (total [K^+] 25.7 mM), basal IP_3 levels were significantly increased (after 30 s: 8.28 ± 1.88 pmol/ mg protein, n = 7; 5.7 mM K^+, 1.79 ± 0.69 pmol/ mg protein, n = 6). Noradrenaline did not stimulate further IP_3 accumulation under these conditions. In contrast, 5-HT (100 μM) caused a transient inhibition of K^+-induced IP_3 accumulation, which was maximal 5 s after the addition of 5-HT (0.98 ± 0.61 pmol/ mg protein, n = 5, 13 % of corresponding basal level) and persisted for at least 15 s before IP_3 accumulation increased after 30 s.

These results suggest that IP_3 is involved in noradrenaline-evoked contraction of the rabbit basilar artery. However 5-HT-induced contraction probably does not involve IP_3, supporting our previous results (Clark & Garland, 1991). Furthermore, raised $[K^+]_o$ stimulates IP_3 accumulation in the rabbit basilar artery, and this increase was transiently inhibited by 5-HT.

Supported by the Wellcome Trust.

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568P CHARACTERIZATION OF α_2-ADRENOCEPTOR SUBTYPE BINDING IN TRANSFECTED CHO CELLS

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Recently, three subtypes of the α_2-adrenoceptor have been characterised and cloned in COS-7 cells (Bylund *et al*, 1992). The purpose of this study was to confirm that the cell lines used did in fact express the reported receptors. Chinese hamster ovary (CHO) cells were obtained from Bylund already transfected with plasmids encoding for the human α_{2A}, rat α_{2B} and opossum α_{2C} receptors. These stable cell lines were grown up in HAMS F12 (containing 1% glutamine and 10% foetal calf serum) at 37°C, before being spun into a pellet and stored in liquid nitrogen until use. A crude membrane preparation was prepared from the cells by homogenisation in TME buffer (50mM TRIS, 10mM $MgCl_2$, 1mM Na-EDTA, pH 7.4) followed by spinning at 47800xg for 15 minutes at 4°C, before being resuspended by homogenisation. Membranes were stored in 5ml aliquots of TME buffer at -80°C until use, at which time they were made up to assay volume and disrupted by sonication. Assays were performed, in triplicate, in 96 well plates in a total volume of 200 µl per well, containing 1nM [^{3}H]rauwolscine, membranes from $4x10^5$ cells, and displacing compounds in concentrations ranging from 10^{-3}M to 10^{-12}M. Following the addition of the tissue, the plates were incubated for 75 minutes at 20°C before being filtered through pre-wetted filters using a Tomtec Mach IIM harvester. The filters were washed with 3x200µl of TME buffer at 4°C, oven-dried and counted on an LKB 1205 Betaplate counter. Data was analysed using Origin (MicroCal Software Inc.). Displacement by oxymetazoline gave IC_{50} values of 11.5±2.5, 2313.1±563.2 and 89.0±32.9 nM for α_{2A}, α_{2B} and α_{2C} respectively (n=3). Displacement by prazosin gave IC_{50} values of 3353.5±281.9, 48.5±11.5 and 1944.9±462.1 nM for α_{2A}, α_{2B} and α_{2C} respectively (n=4). The data for the cells expressing α_{2A} and α_{2B} receptors was in close agreement with previously published work (Harrison *et al*, 1991; Bylund *et al*, 1992; Marjamäki *et al*, 1993). The IC_{50} value obtained for prazosin in the α_{2C}-expressing cells was approximately ten times higher than anticipated, although the IC_{50} for oxymetazoline was close to the published values. A possible explanation for this discrepancy is that the α_{2C} receptors studied in the quoted papers were human-α_{2C4} clones in COS-7 and S115 cell lines, rat RG10 clones in COS-1 cells and α_{2C} receptors occuring naturally in the opossum kidney cell line. It is possible that these receptors differ from those in the transfected CHO cells used for this study. Displacement experiments with additional compounds are currently being performed to provide a more detailed characterization.

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569P EVIDENCE FOR THE PRESENCE OF AN ATYPICAL PREJUNCTIONAL NPY-Y_1 RECEPTOR IN RABBIT ISOLATED VAS DEFERENS: COMPARISON WITH THE RAT VAS DEFERENS

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The prejunctional inhibition of electrically-induced contraction of rat isolated vas deferens by NPY has been proposed to be mediated by the putative Y_2 subtype on the basis of the rank order of potency of NPY, PYY and some of their truncated analogues (Wahlestedt et al.,1986). Recently, benextramine was claimed to be a non-competitive antagonist of postjunctional Y_1 and Y_2 receptor subtypes in rat isolated femoral artery (Tessel et al., 1993) and also to be a selective Y_1 ligand in a rat brain binding assay (Doughty et al., 1992). The aim of this study was to compare the prejunctional effects of NPY and some related peptides in rat and rabbit isolated vas deferens and to test the potential antagonistic activity of benextramine. Prostatic segments of each vas deferens, obtained from Sprague-Dawley male rats (200-300 g) and New Zealand male rabbits (2.0-2.8 Kg), were placed in 2 ml organ bath filled with oxygenated Krebs solution at 33 °C. Electrical field stimulation was performed with single pulses (25 V, 1 ms, 0.15 Hz), delivered through platinum electrodes. Agonists' effect was calculated as % inhibition of the basal contractile response (1.22 ± 0.06 g and 0.52 ± 0.04 g for rabbit and rat respectively; n=45 for each). IC_{50} ± s.e. mean (n=6) and equipotent molar ratios (EPMR) for each agonist are reported in Table 1.

Table 1 IC_{50} ± s.e.mean (expressed in nM, n=6) and EPMR (relative to PYY) exhibited by NPY and some analogues in rat and rabbit isolated vas deferens.

	RABBIT		RAT	
	IC_{50} ± s.e.	EPMR	IC_{50} ± s.e.	EPMR
PYY	1.76±0.11	1	3.77±0.63	1
[Leu^{31},Pro^{34}]NPY	5.04±0.63	2.9	N.S.E.	-
NPY	5.72±0.21	3.2	34.4±3.67	9.1
hPP	6.41±1.05	3.6	N.S.E.	-
NPY_{2-36}	24.0± 2.1	13.6	6.86±0.90	1.8
NPY_{13-36}	1368±20.4	777	166±21	44
NPY_{18-36}	N.D.	-	386±26	102

N.S.E. = No Significant Effect up to 1 µM.
N.D. = Not Determined because max. response at 10 µM was 41.3 ± 4.7 %

Benextramine (100 µM for 10 min) did not increase the IC_{50} for PYY in rat vas deferens (3.59 ± 0.37 nM; n=3) and significantly (P=0.006) decreased the IC_{50} for [Leu^{31},Pro^{34}]NPY in rabbit vas deferens (2.15 ± 0.07 nM; n=3). Increasing the incubation time for benextramine to 60 min gave the same results. In conclusion, the rank order of potency of the agonists seems to indicate that in rat vas deferens NPY prejunctional effects are mediated by a Y_2 receptor subtype insensitive to benextramine. Furthermore, in rabbit vas deferens the high potencies of hPP and NPY_{2-36} and the lack of antagonism by benextramine could indicate the presence of an atypical Y_1 receptor subtype.

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570P CHARACTERISATION OF A PREJUNCTIONAL H_3 RECEPTOR IN THE DOG ISOLATED ELECTRICALLY-STIMULATED SAPHENOUS VEIN

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In 1976, McGrath and Shepherd reported that histamine inhibited the sympathetically-mediated contraction of the electrically-stimulated dog isolated saphenous vein and using the pharmacological tools available, suggested that this inhibition was mediated *via* an H_2 receptor (McGrath & Shepherd, 1976). Recently, a similar prejunctional histamine receptor was described on sympathetic nerve terminals in human isolated saphenous vein (Molderings *et al.* 1992). Using selective H_3 receptor ligands, the receptor in this study exhibited properties that were consistent with the H_3 receptor subtype. Consequently, in the present study, the pharmacological identity of the prejunctional histamine receptor in the canine saphenous preparation has been re-evaluated.

Strips of medial saphenous vein (3mm in width, 20mm in length) were removed from female beagle dogs (8-12kg) and suspended under a load of 0.5g, in Krebs solution gassed with 95% O_2 and 5% CO_2 at 37°C, and field stimulated (2Hz, 0.1msec, 30V) for 10sec every 3min. Unless stated otherwise, all experiments were performed in the presence of indomethacin (1μM), imipramine (1μM), atropine (1μM), mepyramine (1μM) and ranitidine (1μM). A cumulative concentration-effect curve was established for histamine (1nM-10μM) in all tissues. In agonist studies, a second concentration-effect curve was then established for a test compound. In antagonist studies, test compounds were allowed to equilibrate with tissues for 60min before establishing a second curve to either histamine (1nM-10μM) or (R)-α-methylhistamine (1nM-1μM).

Histamine (1nM -10μM) produced a concentration-dependent inhibition of the twitch contraction that was mimicked by ligands which have affinity for the H_3 receptor subtype; N^{α}-methylhistamine (0.1nM-1μM), (R)-α-methylhistamine (1nM-1μM) and (S)-α-methylhistamine (1nM-10μM). The selective H_2 receptor agonist dimaprit was inactive (see below):

Agonist	Histamine	N^{α}-methylhistamine	(R)-α-methylhistamine	(S)-α-methylhistamine	Dimaprit
EC_{50} (nM)	132[59-302]	14[3-68]	22[16-29]	316[109-903]	>1mM
Max inhibition (%)	100	91±7	91±2	100	Inactive
n	12	5	39	4	3

(Data are shown as the geometric mean [95% confidence limits] or the arithmetic mean±s.e.mean for n separate experiments).

In antagonist studies, the concentration-effect curve to (R)-α-methylhistamine was unchanged following the omission of mepyramine (1μM) and ranitidine (1μM) from the bathing medium. However, the selective H_3 receptor antagonist thioperamide (10-300nM) produced a concentration-dependent rightward displacement of the concentration-effect curve to both histamine and (R)-α-methylhistamine (pA_2 and Schild slope values respectively [95% confidence limits] for thioperamide: vs histamine, 7.7[7.6-8.0], 0.9[0.6-1.1], n=15; vs (R)-α-methylhistamine, 8.2[7.9-8.9], 1.0[0.6-1.3], n=14).

In conclusion, the lack of activity of mepyramine, ranitidine and dimaprit suggests that the prejunctional histamine receptor in dog isolated saphenous vein is pharmacologically distinct from either the H_1 or H_2 receptor subtypes. The rank order of agonist potencies, the stereoselectivity exhibited by (R)-α-methylhistamine and (S)-α-methylhistamine, and the high antagonist potency observed to thioperamide suggests that the prejunctional histamine receptor in this preparation is, like that of the human saphenous preparation, of the H_3-subtype.

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571P INHIBITORY EFFECTS OF MUSCARINIC RECEPTOR ACTIVATION ON K^+-EVOKED [^{3}H]NORADRENALINE RELEASE FROM THE HUMAN NEUROBLASTOMA SH-SY5Y

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Activation of muscarinic receptors can inhibit voltage-gated Ca^{2+} channels in sympathetic neurones (e.g. Beech *et al.*, 1991). Here, we have investigated the involvement of Ca^{2+} channels in the release of [^{3}H]noradrenaline ([^{3}H]NA) from the human sympathetic neuroblastoma SH-SY5Y evoked by raised external [K^+], and whether this can be modulated by activation of muscarinic receptors. [^{3}H]NA release was monitored using methods identical to previously described studies (Vaughan *et al.*, 1993), except that phorbol esters were not applied to enhance release. [^{3}H]NA release is expressed as percentage of total [^{3}H]NA content with basal release subtracted. Results presented are means ± s.e.mean and statistical comparisons were made using the unpaired Student's t-test.

Raising external [K^+] from 5mM to 100mM evoked release of 15.1±0.3% (n=44 experiments). This could be reduced to 7.7±0.4% (n=12) by application of 5μM nifedipine ($P<0.00001$), and to 10.8±0.6% by application of 100nM ω-conotoxin GVIA (ω-CgTx; n=22, $P<0.00001$). Application of both nifedipine and ω-CgTx reduced evoked release to 0.82±0.36% (n=4, $P<0.00001$). These findings indicate that K^+-evoked [^{3}H]NA release is largely mediated by Ca^{2+} influx through both L- and N-type Ca^{2+} channels.

Muscarine inhibited K^+-evoked release in a time- and concentration-dependent manner. When applied 2min prior to adding 100mM K^+, 300μM muscarine maximally inhibited release by 87.4±1.6% (n=6, $P<0.00001$), and this inhibition could be completely blocked by 100nM atropine (n=3). A concentration-response curve yielded an IC_{50} value of 1-2μM. When applied simultaneously with 100mM K^+, muscarine (300μM) maximally inhibited by 24.0±2.1% (n=4, $P<0.005$). However, when applied 4min prior to 100mM K^+, 3μM muscarine inhibited evoked release by 81.5±0.8% (n=4, $P<0.00001$).

Veratridine (28μM) evoked [^{3}H]NA release of 4.10±0.04% and this could also be inhibited by 84.1±0.9% (n=4, $P<0.00001$) when 300μM muscarine was applied for 2min previously. In contrast, release (3.3±0.6%) evoked by the Ca^{2+} ionophore A23187 (19μM) was unaffected by 2min pretreatment with 300μM muscarine (n=4).

Our results indicate that K^+-evoked release of [^{3}H]NA from SH-SY5Y cells can be inhibited by activation of muscarinic receptors in a time- and concentration-dependent manner via a mechanism which is likely to involve inhibition of both L- and N-type Ca^{2+} channels.

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572P MYOGENIC TONE IS ASSOCIATED WITH INCREASED INTRACELLULAR CALCIUM IN RAT ISOLATED MESENTERIC AND CEREBRAL SMALL ARTERIES

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The myogenic response is the ability of blood vessels to constrict in response to intravascular pressure elevation (Bayliss 1902). Myogenic tone is abolished by removal of external Ca^{2+}, by calcium channel blockers, and by membrane hyperpolarization, which suggests that it depends on Ca^{2+} influx through voltage-dependent Ca^{2+} channels (Nelson et al., 1990). This study has measured the relationship between myogenic responses and intracellular Ca^{2+} (Ca_i) in small arteries.

3-4mm lengths of rat mesenteric (262±13μm outer diameter) and rabbit cerebral (358±29μm outer diameter) arteries were mounted between micropipettes in a perfusion chamber. The vessels were loaded with the Ca^{2+} indicator, fura-2, by incubating them in modified Krebs physiological salt solution (PSS) containing 5μM fura-2/AM and 1mg/ml bovine serum albumin for 2h at 37°C. Ca_i was measured as the ratio of fluorescence at 510nm evoked by excitation at 340 and 380nm. Changes in diameter and Ca_i (ΔCa_i) are expressed as % response to 120mM K^+. Intravascular pressure steps of 30, 60, 90 mm Hg were applied via a pressure-servo pump. The steps were repeated in PSS and Ca^{2+}-free PSS to distinguish myogenic from passive responses.

Increases in intravascular pressure were associated with a rise in Ca_i and development of myogenic tone. Cerebral arteries tended to show greater myogenic tone and pressure-induced increase in Ca_i than mesenteric arteries (table 1).

Table 1. Changes in diameter and Ca_i induced by pressure steps in isolated mesenteric and cerebral small arteries.

	Mesenteric		Cerebral	
Pressure (mmHg)	Diameter (%)	ΔCa_i (%)	Diameter (%)	ΔCa_i (%)
30	1±7	-	19±6	-
60	8±2	5±1	16±5	12±6
90	-2±2	12±3	12±6	22±7

Data are derived from 3-5 animals and are means ± s.e. means.

These results show that although there appear to be differences in Ca_i between cerebral and mesenteric arteries following intravascular pressure steps these do not seem to be as great as the differences in myogenic tone. This could be due to a difference in sensitivity of the contractile apparatus to Ca_i.

This study was supported by a grant from the British Heart Foundation. ADH is a British Heart Foundation Intermediate Fellow.

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573P UK-84,149: A POTENT AND GUT-SELECTIVE SPASMOLYTIC IN THE ANAESTHETISED DOG

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UK-84,149 is a novel, gut selective spasmolytic which potently displaces radiolabelled diltiazem from cortical membranes and inhibits contraction in gut smooth muscle (Wallis et al, This meeting). We have previously reported that nifedipine only inhibits gastrointestinal motility at doses which produce marked cardiovascular effects. (Quinn et al, 1991) The objective of this study was to assess whether the *in vitro* gut selectivity of UK-84,149 could be confirmed *in vivo* and to compare it with classical L-type calcium antagonists.

Male beagle dogs (11-14.5 kg) were fasted overnight and anaesthetised with chloralose/urethane (0.08 gkg^{-1} 0.8 gkg^{-1}), following premedication with piritramide 4 $mgkg^{-1}$ (Dipidolor). Animals were prepared for measurement of arterial blood pressure, heart rate, cardiac output and left ventricular pressure. Small intestine (SI) and colonic motility were also measured following stimulation by infusion of cholecystokinin octapeptide sulphated (CCK-8s). (Quinn et al, 1991) Compounds were given by infusion and results are expressed as doses ($\mu g.kg^{-1}min^{-1}$) that produce a 50% reduction in motility or total peripheral resistance (TPR) or that increase heart rate to 150% of control or decrease mean blood pressure (MBP) by 25%. Results are expressed as the geometric mean with 95% confidence limits and are summarised below.

	SI Motility ID_{50}	Colon ID_{50}	TPR ID_{50}	Heart Rate ED_{150}	MBP ID_{25}
	mmHg.min	mmHg.min	dyne s^{-1} cm^5	b.p.m.	mmHg
UK-84,149 (n=4)	8.5(5.5-13.5)	6.3(0.9-43)	197(86-450)	>238	>300
Nifedipine (n=4)	1.7(0.6-5.0)	1.1(0.2-5.8)	1.1(0.5-2.7)	1.1(0.6-1.8)	>6.1
Diltiazem (n=4)	80.9(35-186.9)	39.3(1.3-1152)	47.9(10-229.7)	>300	>211

UK-84,149 potently inhibited both CCK-8s stimulated small and large bowel motility, with cardiovascular effects only being evident at doses 10 times higher. This contrasted with L-type calcium channel blockers (including verapamil(n=1)) which produced dose related cardiovascular effects over the same dose range as those required to inhibit motility. Nifedipine at doses up to 10μgkg^{-1} had no adverse effect on A-V conduction, however, this contrasted with both verapamil(10-40$\mu gkg^{-1}min^{-1}$) and diltiazem(10-300$\mu gkg^{-1}min^{-1}$) which both produced dose related increases in P-R interval, with complete A-V block being observed at the top dose. UK-84,149, which like diltiazem, interacts potently with the benzothiazepine site of the calcium channel, had no deleterious effects on cardiac conduction. In conclusion, UK-84,149 is a novel gut selective spasmolytic which exhibits a potent antimotility effect *in vivo* without the cardiovascular effects seen with other calcium antagonists.

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574P UK-84,149: A NOVEL GUT-SELECTIVE SPASMOLYTIC WITH CALCIUM ANTAGONIST ACTIVITY

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Contraction of gastrointestinal smooth muscle is dependent on entry of calcium into the cell and is blocked by L-type calcium channel antagonists, nifedipine, verapamil and diltiazem, but only at concentrations which are active on vascular smooth muscle. In this study we report the profile of UK-84,149, [S-5,11-dihyrdo-5-[1-(4-methoxyphenethyl)2-pyrrolidinylmethyl]-dibenzo[b,e,][1,4]thiazepine maleate], which is a potent gut selective spasmolytic with calcium antagonist activity. Guinea pig ileum and rat aorta experiments were conducted at 32°C in Krebs solution gassed with 95%O_2 and 5%CO_2. Compounds were incubated for 20 mins and the concentration to produce 50% inhibition (-pIC_{50}) of contractions induced by electrical stimulation (supramaximal voltage, 0.1Hz and 0.1msec), acetylcholine, histamine and bradykinin were determined. Also, tissues incubated in calcium free depolarising Krebs (80mM KCl) were contracted with 2mM $CaCl_2$; this contracture was reversed by increasing concentrations of antagonist and a -pIC_{50} was calculated. -pIC_{50} was also determined against contractions (45mM KCl) of the rat aorta. Binding studies with radiolabelled nitrendipine, methoxyverapamil and diltiazem were conducted in bovine and rat cortical membranes respectively at room temperature (Glossman et al, 1985)

Table 1 -pIC_{50} values (mean±S.E.M., n=6-14)

	Rat Aorta		Guinea Pig Ileum			
	K^+ 45mM	Field Stim'n Supra Max	ACh 0.3μM	Histamine 10μM	Ca^{2+} 2mM	Bradykinin 0.2μM
UK-84,149	6.1±0.07	7.5±0.08	7.8±0.07	7.1±0.07	7.2±0.1	7.5±0.1
Nifedipine	8.2±0.09	7.4±0.03	8.1±0.08	8.6±0.16	8.6±0.06	8.4±0.1
Verapamil	6.7±0.07	5.9±0.19	6.9±0.25	7.0±0.15	7.9±0.09	7.2±0.09
Diltiazem	6.4±0.14	5.7±0.14	6.6±0.16	6.4±0.06	7.0±0.15	6.9±0.14

UK-84,149, like the L-type calcium antagonists, was a broad spectrum spasmolytic on the ileum and, significantly, the inhibition of the calcium contracture was reversed by the addition of excess calcium. In order to characterise this calcium antagonist effect further, interaction studies with Bay-K-8644 in the ileum were undertaken. Bay-K-8644 (1-300nM) produced a dose related contraction which was antagonised in an insurmountable manner by UK-84,149 (0.01-10μM), the benzothiazepine, diltiazem (0.01-1μM) and the phenylalkylamine, verapamil (0.1-10μM) . However, this contrasted with the competitive antagonism observed with the dihydropyridine antagonist, nifedipine(0.001-1μM). In radioligand binding studies UK-84,149 (1μM) did not displace [^{3}H]- nitrendipine binding in bovine cortex and only partially displaced [^{3}H]-methoxyverapamil binding at (1μM) in rat cortex. In contrast, UK-84,149 completely displaced [^{3}H]-diltiazem binding in the rat cortex (ED_{50} 45.2nM). These data demonstrate that UK-84,149 is a calcium antagonist, which inhibits contractile responses on the ileum at least 10-fold lower concentrations than on the rat aorta.

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575P THE MOTOR NERVE CONDUCTION DEFICIT IS ATTENUATED IN STREPTOZOTOCIN-DIABETIC RATS BY TREATMENT WITH INOSITOL 1,2,6-TRISPHOSPHATE WITHOUT AN EFFECT ON NERVE BLOOD FLOW

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Acute nerve conduction defects develop early in clinical and experimental *diabetes mellitus*, but they are preceded and arguably derived from a decrease in nerve blood flow (Stevens *et al.*,1994). We have shown previously that treatment of diabetic rats with inositol 1,2,6-trisphosphate (PP56) attenuates the motor nerve conduction (MNCV) deficit (Carrington *et al.*,1993), so this study measured its effect on nerve blood flow. Two large groups of rats were made diabetic by streptozotocin (50 mg/kg i.p.); non-diabetic controls formed a third group. Diabetic rats were given PP56 by continuous subcutaneous infusion from an osmotic mini-pump at 0.1 mg/h exactly as described elsewhere (Carrington *et al.*,1993) throughout the duration of a 4 wk period of diabetes; non-diabetic controls and a parallel diabetic group had implanted mini-pumps delivering normal saline. Pump identities were coded and measurements made blind. At the end of the protocol rats were anaesthetised with either halothane for measurement of MNCV (Carrington *et al.*,1993; sub-group B) or alphadalone: alphaxolone for measurement of sciatic nerve laser Doppler flux and systemic arterial pressure (Stevens *et al.*,1994; sub-group A).

Group	Sub-group A n (rats)	Final body wt (g)	Blood glucose (mmol/l)	Mean arterial pressure (mm Hg)	Nerve Doppler flux (arbitrary units)	Sub-group B n (rats)	MNCV (m/s)
Controls - saline	9	422±28	3.6±0.4	117.8±14.1	386.0±51.1[a]	9	44.9±4.6[a]
Diabetics - saline	10	279±29	27.3±2.8	110.7±7.9	228.4±64.0[b]	10	38.5±3.1[b]
Diabetics - PP56	9	276±18	25.4±3.7	115.5±10.0	260.6±45.3[b]	9	45.0±3.2[a]

Data are mean ± SD; by one-way ANOVA with Duncan's range tests, a *versus* b = $p<0.01$; massive differences were not tested. Diabetes induced hyperglycaemia and weight loss. Neither was affected by PP56 treatment, implying that the drug did not attenuate the severity of diabetes. Neither diabetes nor PP56 affected systemic arterial pressure under anaesthesia, but diabetic rats sustained a marked reduction in sciatic nerve laser Doppler flux, indicating a profound reduction in nerve blood flow. This was not affected by PP56. Diabetic rats given saline only showed a marked reduction in MNCV, which was normalised in the diabetic rats given PP56. These findings demonstrate that, whilst reduced nerve blood flow may be coincident with reduced MNCV, the latter can be normalised without attenuation of the former. This does not disprove cause and effect, but it does indicate that pharmacological correction of neurochemical changes in axon or Schwann cell can correct diabetes-related conduction defects in spite of persistent ischaemia.

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576P CHARACTERIZATION OF THE EFFECTS OF DEPOLARIZING PHOSPHOLIPASES A_2, MYOTOXIN a AND OUABAIN ON THE PERINEURAL WAVEFORMS RECORDED FROM MOUSE MOTOR NERVE ENDINGS

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Toxic phospholipases A_2, Pa-8 (0.2 μM), Pa-10F (0.2 μM) and Pa-11 (0.8 μM), from the venom of king brown snake, *Pseudechis australis* (Takasaki *et al.*, 1990) affected perineural waveforms recorded extracellularly from the pre-terminal part of motor nerve terminals of the mouse triangularis sterni muscle. These toxic enzymes first reduced the second negative deflection of the waveform, which is associated with the efflux of K^+ at nerve terminal. Later, they decreased the first negative spike, which represents Na^+ current at the pre-terminal part of the axon. Since these toxins caused muscle fibre depolarization (Rowan *et al.*, 1989; Fatehi *et al.*, 1993), it was considered that the changes observed in perineural waveforms might result from nerve terminal depolarization. To investigate this hypothesis further, the effects of depolarizing agents, myotoxin a (from the venom of *Crotalus viridis viridis*)(Hong and Chang, 1985), ouabain and KCl on the perineural waveforms have been studied. Myotoxin a (5 μM) decreased the second spike of perineural waveforms by about 30% of control after 45-55 min. Later, after about 60 min, there was also a significant reduction of the initial (I_{Na}) spike. Ouabain (50 μM - 0.5 mM) first reduced the second negative spike and subsequently decreased the first deflection of waveforms in a concentration-dependent manner. Ouabain (50 μM) reduced the second deflection of waveforms by 20 ± 5% but it did not affect the first deflection after 15 min. However, it decreased the first and second deflections by 20 ± 5% and 50 ± 10%, respectively after 30 min. There was no further blockade of signals induced by ouabain at 50 μM even after 1 hour. Ouabain at higher concentrations (0.1 - 0.5 mM) induced a more profound and faster reduction in the second deflection of waveforms followed by a decrease in the first deflection, and eventually ouabain abolished waveforms (after 30 ± 5 min). The effect of KCl (7-12 mM) on the shape of perineural waveforms was very similar to those of ouabain, but the time course of action of Kcl was shorter. The effects of ouabain and KCl on the perineural waveforms were reversible. These observations support the hypothesis that alteration of waveforms by these toxic phospholipases A_2 is mediated by nerve terminal depolarization.

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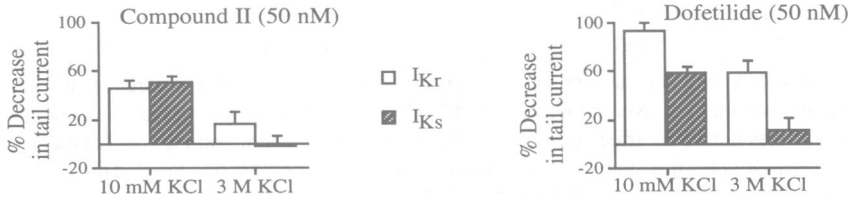
577P THE INFLUENCE OF ELECTRODE CHLORIDE CONCENTRATION ON THE DELAYED RECTIFIER POTASSIUM CURRENT AND ON THE BLOCKING ACTION OF COMPOUND II AND DOFETILIDE

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The effect of microelectrode concentrations of KCl on the delayed rectifier was studied in cardiac ventricle. The block of the delayed rectifier by compound II (Connors *et al.*, 1992) and dofetilide was also investigated with different microelectrode KCl concentrations.

Single cells were isolated from guinea-pig ventricular muscle and delayed rectifier potassium current (I_K) was studied under voltage clamp conditions (balanced salt solution with 2.5 mM calcium; 36°C) and measured as an outward tail current upon repolarization to a holding potential of -40 mV following depolarizations to +40 mV. The current at 40 ms was taken as a measure of the rapid component (I_{Kr}) and that at 400 ms as the slow component (I_{Ks}; Sanguinetti and Jurkiewicz, 1990). Microelectrodes contained BAPTA to suppress the cytosolic calcium transient with: 10 mM KCl + 0.5 M K_2SO_4; 1 M KCl; or 3 M KCl.

I_{Ks} was significantly increased with raised electrode KCl concentration (1 M KCl 564 ± 37 pA, n=5; 3 M KCl 1168 ± 97 pA, n=10) compared with 10 mM KCl / 0.5 M K_2SO_4 electrodes (395 ± 30 pA; n=11; $p<0.01$). The figure shows the blocking effect of 50 nM compound II and 50nM dofetilide with 10 mM and 3 M KCl electrodes. While compound II reduced I_{Kr} by 46 ± 6 % (n=5) and I_{Ks} by 51 ± 5 % with 10 mM KCl electrodes, there was no significant change with 3 M KCl electrodes in either component. Thus, the effect of compound II with 3 M KCl electrodes was significantly reduced compared to 10 mM KCl electrodes (I_{Kr} $p<0.05$; I_{Ks} $p<0.01$). Dofetilide reduced I_{Kr} by 93 ± 4 % with 10 mM KCl electrodes compared to 59 ± 6 % with 3 M KCl electrodes and I_{Ks} by 59 ± 5 % with 10 mM KCl electrodes whereas no significant change was observed with 3 M KCl electrodes. Therefore, the effect of dofetilide with 3 M KCl electrodes was also significantly reduced compared to 10 mM KCl electrodes (I_{Kr} $p<0.01$; I_{Ks} $p<0.01$; n=5-7).



The increase in I_K observed with high KCl may reflect a chloride regulation of I_K (Carmeliet and Verdonck, 1977) which modifies the blocking action of both compound II and dofetilide. The mechanism and therapeutic significance of this remains for future study.

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This work was supported by the British Heart Foundation.

578P EVIDENCE FOR SUBDIVISION OF P_{2U}-PURINOCEPTORS BASED ON SURAMIN SENSITIVITY

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UTP has a range of effects in many diverse tissues e.g. relaxation and/or contraction in vascular preparations, increasing $[Ca^{2+}]_i$ in cell lines such as HL60 cells. Although the structure of a "P_{2U}-receptor" has recently been elucidated (Lustig *et al.*, 1993) no selective antagonists have so far been discovered which show any appreciable selectivity for this receptor. Suramin has been shown to be a non-selective antagonist at P_{2X}- and P_{2Y}- purinoceptors (Hoyle *et al.*, 1990) whilst showing little if any antagonism of P_{2U}-receptors in bovine aortic collateral arteries (Wilkinson *et al.*, 1994). The purpose of this study was to determine the effects of suramin at P_{2U}-receptors in the rat aorta (Dainty *et al.*, 1991) and canine tracheal epithelium (Pollard & Roberts, unpublished observations).

P_{2U}-receptor mediated increases in chloride secretion by canine tracheal epithelium were measured using the Ussing chamber technique. All agonists were added to the apical side of the tissue. P_{2U}-receptor mediated endothelium-dependent relaxations of pre-constricted rat aortic rings were tested using standard organ-bath techniques. Cumulative concentration response curves to ATP, UTP and the stable analogue ATPγS were constructed in both preparations in the absence and presence of 100μM suramin. Potency ($p[A_{50}]$) estimates were obtained by logistic curve-fitting.

In the rat aorta but not the canine epithelium, suramin caused statistically significant rightward shifts in subsequent E/[A] curves to ATP, UTP and ATPγS, see Table 1.

Table 1.

	Rat aorta		Canine epithelium	
	Control	+ 100μM suramin	Control	+ 100μM suramin
ATP	5.71 ± 0.08	4.78 ± 0.09 *	6.1 ± 0.10	6.3 ± 0.03 NS
UTP	6.36 ± 0.07	5.64 ± 0.07 *	6.7 ± 0.10	6.6 ± 0.13 NS
ATPγS	6.17 ± 0.09	5.53 ± 0.11 *	5.8 ± 0.03	5.6 ± 0.10 NS

$p[A_{50}]$ (mean ± s.e., n=4-9). Comparing $p[A_{50}]$s ± suramin: * = $P<0.05$, NS = $P>0.05$, Students paired *t* test.

The results of this study provide evidence for subdivision of P_{2U}-receptors based on their sensitivity to suramin.

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579P SR33589, A NEW AMIODARONE-LIKE ANTIARRHYTHMIC AGENT: ELECTROPHYSIOLOGICAL EFFECTS IN DOGS

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SR 33589 is a new agent structurally similar to amiodarone known to prolong monophasic action potential duration, inhibit adrenergic stimulation and reduce arrhythmias (Bruyninckx et al., 1992; Manning et al., 1992). We have compared its effects to amiodarone on atrial, AV nodal and ventricular effective refractory periods (ERP's), and sinus cycle (SCL) and Wenckebach cycle (WCL) length. Mongrel dogs were anaesthetized with chloralose (80 mg/kg i.v.) and electrodes implanted into the right atrium for stimulation of the SA node and recording the atrial electrogram. Electrodes were implanted also in the right ventricle for measurement of the His bundle potential and stimulation of the ventricle. 2.5 and 5 mg/kg SR 33589 iv prolonged atrial ERP by 17 and 19%, AV nodal ERP by 63 and 105% ($P<0.001$) and ventricular ERP by 15 and 22% ($P<0.01$). WCL was prolonged by 44 and 83% and SCL by 29 and 48% ($P<0.01$). 5 mg/kg iv amiodarone prolonged atrial ERP by 13% ($P<0.05$), AV nodal ERP by 19% and ventricular ERP by 11% ($P<0.01$). WCL and SCL were prolonged by 31% ($P<0.01$) and 26% (N.S.). HV interval was not significantly altered by either agent, whilst AH interval was prolonged significantly by both doses of SR 33589.

Table 1. Effect of amiodarone and SR 33589 on electrophysiological indices in anaesthetized dogs. (% change)

	Sinus cycle length	Effective refractory period			Wenckebach cycle length	AH interval	HV interval
		Atrial	AV node	Ventricular			
Amiodarone							
5 mg/kg iv	26	13*	19*	11**	31**	13	12
SR 33589							
2.5 mg/kg iv	29**	17***	63**	15**	44***	24**	4
5.0 mg/kg iv	48**	19	105***	22**	83***	60**	12

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus pre-drug values (n = 6 in each group), calculated using a paired t-test.

Thus the electrophysiological profile of intravenous administration of SR 33589 in dogs is very similar to that of amiodarone but its effects are apparent at lower doses.

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580P CLASSIFICATION OF ADENOSINE A_1 RECEPTORS BY AGONIST POTENCY ORDERS IN THE SINOATRIAL AND ATRIOVENTRICULAR NODES OF THE GUINEA-PIG

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The rank order of agonist potency for adenosine (ADO) A_1-receptors mediating negative chronotropic and inotropic effects in the heart has been reported as follows: R-N^6-(2-phenylisopropyl)adenosine (R-PIA) = N^6-cyclohexyladenosine (CHA) = 5-N-ethyl-carboxamidoadenosine (NECA) > 2-chloroadenosine (CADO) > S-PIA (Ribeiro & Sebastiao, 1986). The ADO receptors in the atrioventricular (AV) node have also been reported to be of the A_1-subtype on the basis of antagonist and agonist studies (Belardinelli *et al.*, 1989). However, as far as we are aware, a comparative study of agonist potency order between the sinoatrial (SA) and AV nodes has not been reported. As part of a study to explore the degree of ADO-receptor homogeneity in the SA and AV nodes, the agonist potencies of seven ADO analogues have been studied in both tissues.

Isolated right atria (guinea pigs 300-400g) were suspended in 20 ml organ baths in Krebs-Henseleit buffer (gassed with 95% O_2/5% CO_2) at 37°C and isolated hearts (guinea pigs 200-300g) were perfused with Krebs solution at 35°C. Single agonist concentration-effect curves were obtained for NECA, CHA, R- and S-PIA, CADO, N^6-methyladenosine (MeAdo) and 2-phenylaminoadenosine (CV 1808). Responses from the right atria and isolated perfused hearts were expressed as absolute changes in rate (Δ basal beats min^{-1}) and SR-interval (Δ SR-interval: ms), respectively.

Table 1. Comparison of the potency of ADO-receptor agonists in the SA and AV nodes of the guinea pig.

Compound	*SA node (log [EC_{50}])*	*AV node (log [EC_{50}])*
NECA	-7.21 ± 0.08	-6.82 ± 0.10
R-PIA	-6.86 ± 0.08	-6.86 ± 0.05
CHA	-6.83 ± 0.02	-6.92 ± 0.08
CADO	-6.62 ± 0.10	-6.49 ± 0.07
S-PIA	-5.62 ± 0.05	-5.50 ± 0.10
CV 1808	-4.57 ± 0.10	-4.41 ± 0.07
MeAdo	-4.35 ± 0.04	-4.60 ± 0.05

(Mean ± s.e.mean; n=5/6)

There were no significant differences in the rank order of agonist potency, which was CHA = R-PIA = NECA ≥ CADO > S-PIA > CV 1808 = MeAdo. These results are consistent with the presence of a homogeneous population of ADO A_1-receptors in both tissues. Furthermore, linear regression analysis of the data gave slope and y-intercept values of 1.05 ± 0.08 and 0.23 ± 0.48, respectively. This indicates that there are no differences in the efficacy of the agonists which, at its simplest, could reflect equal receptor density in the guinea-pig SA and AV nodes.

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581P SURAMIN INHIBITS THE ANGIOGENIC ACTIVITY OF VASCULAR ENDOTHELIAL GROWTH FACTOR *IN VIVO*

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Vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) is a heparin-binding endothelial cell-specific mitogen with angiogenic activity *in vivo* (Ferrara *et al.*, 1992). Suramin is an effective analogue of heparin/heparan sulphate which inhibits the activities of several heparin-binding growth factors such as transforming growth factor-beta, fibroblast growth factor and platelet-derived growth factor. Here we use a rat sponge model to examine the angiogenic activity of $VEGF_{165}$ and its blockade by suramin. The action of suramin on another endothelial cell-specific angiogenic factor, platelet-derived endothelial cell growth factor (PD-ECGF), was also studied.

Sterile polyether sponges with attached cannulae were implanted subcutaneously in male Wistar rats (180-200 g) and neovascularisation was assessed by using ^{133}Xe clearance to measure blood flow through the implants every two days over a period of 14 days. Test substances (50 µl) were injected to the sponges through the cannulae daily. To exclude acute vascular effects of these peptides, they were applied 24 h before ^{133}Xe measurement.

Daily doses of 250 ng of $VEGF_{165}$ caused an intense neovascularisation. The 6 min ^{133}Xe clearance value (all are mean ± s.e.m.) of the control group on Day 6, 8, 10 were 13.6±1.1, 20.0±1.1, 25.2±0.7 % (n = 12) while $VEGF_{165}$ increased it to 22.9±0.5, 33.0±1.5, 44.2±2.5 %, respectively (n = 6, P < 0.01). The $VEGF_{165}$-induced neovascular response was inhibited by co-injection of 3 mg suramin or a polyclonal anti-$VEGF_{165}$ antiserum ($VEGF_{AS}$; 1:100). The ^{133}Xe clearance values on Day 6, 8, 10 for the co-administration groups were 14.6±0.5, 19.2±1.0, 26.5±2.3 % (n = 6, P < 0.05) and 12.9±1.5, 23.0±2.1, 27.1±3.4 % (n = 6, P < 0.05), respectively. Histological studies of sponge sections showed that both the cellular infiltration and neovascularisation in the $VEGF_{165}$-treated sponges were profoundly suppressed by suramin and $VEGF_{AS}$. Lower doses (25 ng) of $VEGF_{165}$ produced no apparent effect on sponge-induced neovascularisation.

Daily administration 450 ng PD-ECGF also caused an intense neovascularisation. The 6 min ^{133}Xe clearance values obtained on Day 6, 8, 10 for the PD-ECGF group and the control group were 20.2±1.0, 28.9±1.8, 36.7±1.5 % and 15.0±0.6, 20.2±1.2, 29.9±1.2 %, respectively (n = 12, P < 0.01). ^{133}Xe clearance data and histology showed that the PD-ECGF-induced neovascular response was not inhibited by co-injection of 3 mg suramin. However, it was inhibited by co-administration of a rabbit polyclonal neutralising antiserum to PD-ECGF (PD-$ECGF_{AS}$; 25 µg day^{-1}). The ^{133}Xe clearance values on Day 6, 8, 10 for the co-administration of antiserum group was 14.8±1.5, 19.5±1.0, 31.0±1.5 % (n = 6, P < 0.05).

Thus, our data suggest that suramin or its analogues are effective antagonists of the *in vivo* action of heparin-binding angiogenic factors such as VEGF, but not non-heparin binding growth factors like PD-ECGF. This study also suggests that VEGF and PD-ECGF elicit angiogenesis by distinct mechanisms. Indeed, we have recently shown that thymidine phosphorylase activity is an integral part of the angiogenic activity of the PD-ECGF molecule (Fan *et al.*, 1994). Further studies are necessary to delineate the exact role of these growth factors in vascular development and to explore the therapeutic potential of their antibodies and antagonists.

This work is supported by the Wellcome Trust.

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582P POTENTIATING INTERACTION BETWEEN ADENOSINE AND 5-N-ETHYLCARBOXAMIDOADENOSINE IN THE GUINEA-PIG ATRIOVENTRICULAR NODE

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Adenosine (ADO) and 5-N-ethylcarboxamidoadenosine (NECA) exert negative dromotropic effects in the atrioventricular (AV) node of the guinea pig by acting on ADO A_1-receptors (Belardinelli *et al.*, 1989; Meester *et al.*, 1994). In preliminary experiments, we suspected that ADO was potentiating NECA, when we had expected that the interaction between two agonists competing for the same receptors should move the underlying concentration-effect curve to the right. We have investigated this phenomenon by obtaining ADO and NECA curves with and without ADO transport blockade by dipyridamole (DIP) (Meester *et al.*, 1993) and in the absence and presence of fixed concentrations of each other.

Isolated hearts from male guinea pigs (200-300g) were perfused with Krebs-Henseleit buffer (gassed with 95% O_2/5% CO_2; 35°C). Single agonist concentration-effect curves were obtained in the absence and presence of the test drugs after an incubation period of 30 min. Responses were expressed as absolute changes in SR-interval (Δ SR-interval: ms). The results were compared with earlier experiments in which the effect of DIP was tested on ADO and NECA curves.

Table 1. Potentiating interaction between ADO and NECA in the guinea-pig AV node

Agonist	Treatment	Control *log [EC_{50}]*	Treated *log [EC_{50}]*	log dose ratio
ADO	1 μM DIP	-5.12 ± 0.15	-5.90 ± 0.09	0.78 ± 0.17*
NECA	3 μM DIP	-6.47 ± 0.06	-6.59 ± 0.07	0.12 ± 0.09
ADO	0.1 μM NECA	-4.52 ± 0.08	-5.06 ± 0.11	0.54 ± 0.14*
NECA	10 μM ADO	-6.70 ± 0.05	-7.17 ± 0.09	0.47 ± 0.10*
ADO	1 μM DIP/0.1μM NECA	-6.62 ± 0.05	-6.74 ± 0.04	0.12 ± 0.06

(Mean ± s.e.mean; n=4-6; * $p<0.05$)

The results show that NECA, the potency of which is not affected by the presence of the uptake inhibitor DIP, potentiates the action of ADO only when the uptake process is not blocked by DIP. We cannot account for these data in terms of NECA and ADO acting solely at a common extracellular receptor. One possible explanatory model would involve ADO expressing an additional intracellular action which potentiates the extracellular receptor responses.

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583P RENAL VASCULAR REACTIVITY IN ACUTE RENAL FAILURE

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Some forms of experimental acute renal failure (ARF) respond to treatment with adenosine antagonists (Kellett et al 1989), indicating a significant role for adenosine in their pathophysiology; but by contrast, in other types of ARF adenosine appears to be of little or no importance since adenosine antagonists have no beneficial effects (Rossi et al 1990). The reason why adenosine is important in some forms of ARF, but not in others, may be related to alterations in sensitivity to the renal vasoconstrictor effects of adenosine. Thus, the aim of this study was to investigate this possibility in rats with ARF induced with glycerol (ameliorated by adenosine antagonists) or mercuric chloride, $HgCl_2$ (unresponsive to treatment with adenosine antagonists).

Male Wistar rats received either 50% v/v glycerol in saline (10 ml kg^{-1} i.m.) or $HgCl_2$ (2 mg kg^{-1}, 2 ml kg^{-1} s.c.) with control animals receiving equivalent volumes of saline. At various times following induction of ARF, rats were anaesthetised, a flow probe placed on the left renal artery and a cannula inserted in the left common iliac artery such that its tip was positioned just rostral to the exit of the left renal artery from the aorta. Falls in renal blood flow (RBF) were recorded in response to bolus injections of adenosine (0.01-10 μg) or noradrenaline (0.001-0.1 μg). Forty eight hours after glycerol injection, RBF was 4.1 ± 0.3 (mean ± s.e.mean, n=8) ml min^{-1} compared to 5.4 ± 0.6 ml min^{-1} (n=8) in controls. The dose-response curve to adenosine in rats with glycerol-induced ARF, 48h following injection, was displaced to left in a non-parallel manner in comparison to the curve for controls. With the exception of the 0.01 μg dose of adenosine, the % falls in RBF in glycerol-injected rats were significantly ($P<0.05$, Student's *t*-test) greater to all doses of adenosine compared to equivalent responses in controls. For example, 10 μg of adenosine evoked a 62 ± 4% (n=8) fall in RBF in animals injected with glycerol in comparison to a 27 ± 5% (n=8) decrease in RBF in controls. By contrast, there was no significant change in the renal vascular response to noradrenaline. Forty eight hours after $HgCl_2$ injection, RBF was 4.5 ± 0.5 (n=8) ml min^{-1} compared to 4.9 ± 0.7 (n=8) ml min^{-1} in controls. Rats injected with $HgCl_2$ showed a modest increase in sensitivity to the renal constrictor action of adenosine, such that 10μg of adenosine produced falls in RBF of 41 ± 10% (n=8) and 30 ± 8% (n=8) in $HgCl_2$-treated rats and saline controls, respectively. However, at no dose did these differences attain statistical significance ($P>0.05$). $HgCl_2$ treatment resulted in no statistically significant change in the renal constrictor responses to noradrenaline. Renal constrictor responses to adenosine were also determined 30 min and 16h following glycerol injection. At 30 min there was no change in sensitivity whereas at 16h there was a moderate increase in responsiveness (37 ± 7% n=8 and 23 ± 5% n=8 falls in RBF produced by 10μg of adenosine in glycerol and saline-injected rats respectively). Although the response to 10μg at 16h was not statistically significant ($P>0.05$), the renal constrictor responses to 1 and 3 μg of adenosine were significantly greater than control responses ($P<0.05$).

The results demonstrate that during the development of ARF induced by glycerol, but not by $HgCl_2$, there is significant and selective increase in sensitivity to the renal vasoconstrictor actions of adenosine. Such an enhanced vascular responsiveness to adenosine may determine, at least in part, whether a particular form of ARF responds to treatment with adenosine antagonists.

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Rossi, N., Ellis, V., Kontry, T., Gunther, S., Churchill, P. & Bidani, A. (1990) *Am. J. Physiol.* **258**, F1554-F1560

584P IMPROVEMENT OF CARDIAC FUNCTION FOLLOWING CHRONIC ANTIHYPERTENSIVE TREATMENT OF SPONTANEOUSLY HYPERTENSIVE RATS WITH CAPTOPRIL PLUS HYDROCHLOROTHIAZIDE

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The effects of chronic (6 months), oral treatment with captopril (36 mg kg^{-1} day^{-1}) plus hydrochlorothiazide (18 mg^{-1} kg^{-1} day^{-1}) on cardiac function and mass were evaluated in 3-month old, male spontaneously hypertensive rats (SHR). Normotensive WKY received no drug. (n = 10 per group). At 9 months of age systolic arterial pressure was measured in unanesthetized rats (Chillon *et al.*, 1992), then systemic arterial compliance (Liu *et al.*, 1986), peripheral resistance and cardiac performance (= peak stroke volume / cardiac mass; Pfeffer *et al.*, 1979) were measured in pithed rats. Cardiac mass was determined as left ventricular weight (mg) expressed relative to body weight (kg).

	SHR	SHR	WKY
treatment	none	captopril + hydrochlorothiazide	none
Systolic arterial pressure (mmHg)	225±3	152±2*	150±2*
Systemic arterial compliance (µl $min^{-1}mmHg^{-1}$)	5.41±0.32	6.77±0.42*	6.84±0.34*
Peripheral resistance (mmHg ml^{-1} min^{-1})	1.79±0.10	0.95±0.10*	1.21±0.08*
Cardiac performance (µl $beat^{-1}$ g^{-1})	307±26	479±48*	488±75*
Cardiac mass (mg kg^{-1})	3247±121	2496±69*	2696±100*

Results are given as means ± s.e.mean; * = $p < 0.05$ vs untreated SHR. Analysis of variance and Scheffé test.

Chronic treatment of SHR with captopril plus hydrochlorothiazide reduces cardiac mass and improves cardiac performance. Multiple regression analysis revealed that both peripheral resistance and systemic arterial compliance were significant determinants of cardiac performance and hypertrophy.

In conclusion, chronic treatment of SHR with captopril plus hydrochlorothiazide corrected the resistance and capacitance components of cardiac afterload, leading to regression of left ventricular hypertrophy and improvement of cardiac performance.

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585P ROLE OF OXIDATIVE PHOSPHORYLATION IN CALCIUM HOMEOSTASIS AND CHANGES IN PERFUSION PRESSURE FOLLOWING STIMULATION WITH NORADRENALINE IN THE RAT TAIL ARTERY

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In order to evaluate the relative role of oxidative phosphorylation in noradrenaline (NA, 1 µM, 2 min) - induced vasoconstriction, changes in intracellular calcium ($\Delta[Ca^{2+}]_i$, arbitrary units, Grynkiewicz *et al.*, 1985) and perfusion pressure (mmHg), and tissue levels of ATP (µmol/g protein) were measured *in vitro* in perfused rat tail artery segments preloaded with Fura-2/AM (Sigma Chemical Co., St. Louis, Missouri, USA; 5 µM, 90 min, Capdeville-Atkinson *et al.*, 1993). NA was applied 7 times at 9 min intervals. 2,4 dinitrophenol (DNP, 40 µM), a specific inhibitor of oxidative phosphorylation, was added from the 2nd to the 6th application. ATP was measured using the luciferin bioluminescent method (Lundin *et al.*, 1976). Results were expressed as mean ± s.e.mean (n = 6 / series).
NA produced an initial short-lived peak in $[Ca^{2+}]_i$, followed by a plateau; the vasoconstrictor response was monophasic. Blockade of ATP production did not modify calcium mobilization (peak and plateau) (Figure 1) but greatly diminished the vasoconstrictor response and tissue levels of ATP. Removal of DNP restored the vasoconstriction but not the level of ATP (Figure 2, * = $P < 0.05$ versus control; analysis of variance and Scheffé test; control: open symbols; DNP: full symbols).

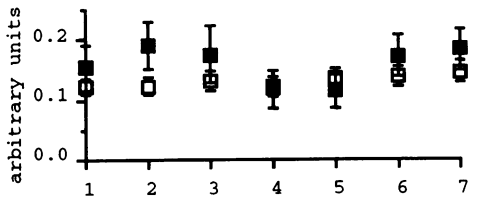
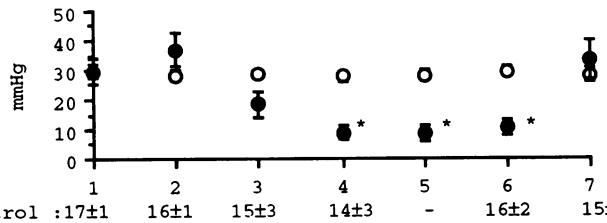


Figure 1. Effect of DNP on NA-induced $\Delta[Ca^{2+}]_i$ peak.



	1	2	3	4	5	6	7
[ATP]control	:17±1	16±1	15±3	14±3	-	16±2	15±2
[ATP]DNP	:17±1	19±3	8±2*	5±2*	-	3±1*	6±1*

Figure 2. Effect of DNP on (1) NA-induced increase in perfusion pressure, and (2) ATP levels ([ATP]).

Our results suggest that (1) calcium homeostasis is independent of oxidative phosphorylation, and (2) oxidative phosphorylation maintains an ATP store (with a rapid turnover) which, in turn, is essential for the NA-induced vasoconstriction.

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586P CHRONIC ANGIOTENSIN-CONVERTING ENZYME INHIBITION COUNTERACTS THE STRUCTURAL AND FUNCTIONAL CONSEQUENCES OF ARTERIAL CALCIUM OVERLOAD

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We investigated the effect of angiotensin-converting enzyme inhibition on the consequences of arterial calcium overload, induced by treating young rats with vitamin D (300,000 IU, i.m.) plus nicotine (2 X 25 mg/kg, p.o.; VDN; Fleckenstein *et al.*, 1989). VDN rats received the angiotensin-converting enzyme inhibitor, perindopril (1 mg/kg/day), or placebo, orally. Ninety days later, systemic arterial compliance was measured in pithed rats (Liu *et al.*, 1986). Aortic calcium content and histomorphometric parameters (aortic internal diameter and percentage of elastin in the media) were measured.
Results are expressed as means ± s.e.mean, n = 12 per group.

Results	controls	VDN	VDN
Treatment	placebo	placebo	perindopril
Aortic calcium content (µmol/g)	8.2 ± 1.0	337.0$ ± 104.9	114.5$* ± 39.1
Aortic internal diameter (mm)	0.97 ± 0.05	1.36$ ± 0.06	1.15* ± 0.06
% elastin in the media	43.4 ± 1.1	39.1$ ± 1.6	45.2* ± 1.9
Systemic arterial compliance (µl min^{-1} $mmHg^{-1}$)	6.69 ± 0.39	5.41$ ± 0.28	7.51* ± 0.68

$ = P<0.05 versus controls and * = P<0.05 versus VDN rats treated with placebo (analysis of variance and Scheffé test).

In VDN rats, calcium deposition produced a fall in the percentage of elastin suggesting a breakdown of the elastic network, leading to dilatation and a decrease in systemic arterial compliance (Atkinson *et al.*, 1994). Chronic angiotensin-converting enzyme inhibition was "anti-calcinotic" in that it attenuated calcium overload and maintained the integrity of vascular structure and function.

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587P SIMULTANEOUS MEASUREMENT OF INTRACELLULAR CALCIUM LEVELS AND THE DAMPING CAPACITY OF THE RAT COMMON CAROTID ARTERY IN A NEW *IN VITRO* PULSATILE SYSTEM

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In vivo the arterial wall is subjected to both static and phasic changes in transmural stress. However, most of available methods for the study of arterial compliance are designed to evaluate the effect of changes in static mechanical stresses only on arterial dimensions.

Carotid artery segments (1.4-cm long) from male Wistar rats (450-500g, n=5) were mounted vertically in a spectrofluorometer (Fluorolog F1T11, Spex, Edison, NJ, USA) and connected to a pulsatile pressurization system consisting of a peristaltic pump and an adjustable silicone tubing resistance. Carotid mean intraluminal pressure was monitored by two pressure transducers placed before and beyond the artery. The carotid damping capacity was estimated from the ratio of the pulsatile pressure signals. The segments were subjected to four rapid pressurization cycles, then to increases in mean intraluminal pressure of 25 mmHg at 5-min intervals from 25 to 150 mmHg (Table 1). A similar pressurization cycle was also performed on a rigid polyethylene tubing (n=7) and on a carotid artery segment following formaldehyde fixation (n=4).

Table 1. Index of carotid damping capacity relative to mean intraluminal pressure.

Mean pressure (mmHg)	25	50	75	100	125	150
Damping capacity x100						
Carotid artery	35±2	33±1	55±4	96±4	104±3	102±1
Carotid artery after fixation	106±5	101±3	106±5	103±3	102±4	104±3
Tubing	108±2	108±3	105±2	105±2	107±3	103±2

Results are given as means ± s.e.mean (analysis of variance and Scheffé test)
In a second experiment, carotid segments were loaded with Fura-2/AM (Sigma Chemical Co., St. Louis, Missouri, USA; 5 µM) for 105 min at 25 mmHg, and washed with Krebs solution for 30 min. Noradrenaline (10 µM) was superfused for 10 min. The fluorescent signals were recorded at 340 or 380 nm (excitation) and 510 nm (emission) and the fluorescence technique was adapted from Capdeville-Atkinson *et al.* (1993). Before, during and after noradrenaline stimulation, the damping capacity was 37±4, 58±6*, 39±4 and intracellular calcium concentration was 82±15, 150±37*, 80±12 nM, respectively (*P<0.05 *versus* initial value). During this time, mean intraluminal pressure remained stable (22.2±1.5, 21.5±1.8, 21.8±1.8 mmHg, respectively).

In conclusion our method allows the simultaneous measurement of the response of the carotid artery to phasic changes in stress and intracellular calcium concentration. The damping capacity was maximal at intraluminal mean pressures lower than 100 mmHg and was reduced by noradrenaline stimulation, with an increase in intracellular calcium concentration, but no alteration in mean intraluminal pressure.

Capdeville-Atkinson, C., Oster, L., Thorin-Trescases, N. *et al.* (1993) *Am. J. Physiol.* 265: C1689-C1702.

588P INVESTIGATION OF DEXAMETHASONE EFFECTS ON LEUCOCYTE BEHAVIOUR USING THE HAMSTER CHEEK POUCH TECHNIQUE

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This study aimed to observe the effect of dexamethasone (DEX) on leucocyte rolling and adhesion by means of an intravital microscopy tecnique. Although steroids are the most potent anti-inflammatory agents which affect leucocyte migration in a variety of experimental systems few studies have focussed on their effect upon rolling and adhesion of white blood cells.

Hamster cheek pouches were prepared as described by Duling (1973). Male golden hamsters (90-100 g) were anaesthetised with pentobarbitone (60 $mgkg^{-1}$ i.p.) and cheek pouches everted, cut longitudinally, the connective tissue removed, and extended in a plastic chamber. Pouches were superfused with bicarbonate-buffered solution (pH=7.4, 5% CO_2/95% N_2 at 5 $mlmin^{-1}$) and equilibrated for a 30 min period. Post-capillary venules with a diameter of 20-30 μm and a segment of at least 100 μm length were chosen for analysis. Formyl-Met-Leu-Phe (FMLP) was topically applied at the dose of 10 nmol (in 50 μl) and images recorded for 30 min. Animals received either saline or DEX 1 $mgkg^{-1}$ s.c. 2h prior to application of FMLP. A Zeiss Axioskop microscope equipped with a water immersion 40X objective was used. A videocamera (Panasonic) connected to a recorder (Panasonic) and a colour monitor (Sony) were used for recording and off-set analysis. Data were analysed as i) velocity of leucocytes ($\mu msec^{-1}$) which rolled on endothelium ; ii) number of rolling cells (cut off speed of 10 $\mu msec^{-1}$); iii) number of cells adherent to the same site for more than 30 sec; iv) number of adherent cells which returned to the blood stream; and v) time necessary to begin migration. The efficacy of the DEX treatment protocol was assessed separately in a zymosan peritonitis model, where hamsters received either saline or DEX (0.3-3 $mgkg^{-1}$ s.c.) 2h prior to challenge with zymosan (2 mg i.p.). Migrated neutrophils were counted at the 4h time point by staining of lavage fluid in Turk's solution. Statistical differences were evaluated by ANOVA, followed by the Bonferroni test, or by Fisher's exact test.

DEX 1 $mgkg^{-1}$ inhibited zymosan-elicited 4h-neutrophil migration by 55%: 29.0±3.0 and 13.1±3.1 $x10^6$ neutrophils in control and DEX-treated hamsters (n=6, $p<0.01$). Microscopic analysis showed that this dose of the steroid did not modify leucocyte behaviour under control conditons (not shown). FMLP reduced the velocity of leucocytes from 20.7±1.8 to 6.9±0.5 $\mu msec^{-1}$ (n=6 hamsters, $p<0.01$) and increased the number of rolling cells from 1.5±0.4 to 5.8±0.6 (n=6, $p<0.01$) with a maximal effect at 0-5 min. The number of adherent leucocytes was also increased from 0.1±0.2 to 9.5±0.7 in the entire 30 min of observation. Treatment with DEX (1 $mgkg^{-1}$) did not modify the parameters reported above. However, the steroid altered the fate of adherent leucocytes in that 73% of cells returned to the blood stream after 2.2±0.2 min (whereas in control animals, only 22% of adherent leucocytes did so, $p<0.01$). Moreover, in saline-treated animals FMLP-induced adherent cells began to migrate in 3.6±0.2 min (n=29 cells of 6 hamsters), this time being increased to 13.7±0.9 min after treatment with DEX (n=9 cells of 6 animals, $p<0.01$).

This study shows that DEX affects leucocyte behaviour once cells are adherent to the endothelial wall by increasing the rate of detachment and the time for the cells which remain adherent. The steroid may interfere with specific adhesion molecules and/or with the activation process of the cell which follows adhesion: future experiments will explore these possibilities.

The financial support of ONO Pharm. Co. (Japan) is greatly acknowledged. RJF is funded by an endowment of Lilly UK.

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589P DIFFERENTIAL SENSITIVITY OF PAF-INDUCED EOSINOPHILIA AND LPS-INDUCED NEUTROPHILIA TO DEXAMETHASONE IN GUINEA-PIG LUNG

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We have previously shown that aerosols of the β_2-adrenoceptor agonist, salmeterol, inhibit platelet activating factor (PAF)-and lipopolysaccharide (LPS)-induced granulocyte accumulation in the lung with similar potency (Whelan and Johnson, 1992; Whelan *et al*, 1993). In the present study we have investigated the effect of dexamethasone on PAF-induced eosinophilia and LPS-induced neutrophilia in guinea-pig lungs.

PAF-induced eosinophilia and LPS-induced neutrophilia in guinea-pig lung were induced and evaluated as described previously (Whelan & Johnson,1992). All data for cell counts (cells.μl^{-1} bronchoalveolar lavage fluid, BALF) are presented as mean ± s.e.mean of the number of observations shown in parentheses.

Twenty four hours after inhalation of PAF (nebuliser concentration 100 $\mu g.ml^{-1}$), BALF eosinophil numbers were increased from 16.3± 1.4 (n=15) to 132.7±11.5 (n=15) cells.μl^{-1}. Dexamethasone (0.5-50 $mg.kg^{-1}$ i.p., 3h before PAF) caused a dose-related inhibition of PAF-induced eosinophilia such that a dose of 1.5 $mg.kg^{-1}$ significantly ($P<0.05$) reduced the BALF eosinophil count to 77.0±9.1 cells. μl^{-1} (n =15), a decrease of 48%. Four hours after inhalation of LPS (100 $\mu g.ml^{-1}$), BALF neutrophil numbers increased from 6.6±1.7 (n=14) to 384.0±60.0 (n=12) cells.μl^{-1}. Dexamethasone (5 to 150 $mg.kg^{-1}$ i.p., 3h before LPS) caused a dose-related inhibition of LPS-induced neutrophilia and at a dose of 15 $mg.kg^{-1}$, the neutrophil count was significantly ($P<0.05$) reduced by 55% to 175.0±26.0 cells.μl^{-1} (n=13).

These data show that dexamethasone (i.p.) inhibits both PAF-induced eosinophilia and LPS-induced neutrophilia in the lung, although, a ten fold greater dose is required to inhibit neutrophil accumulation than eosinophil accumulation. These findings suggest that the cells involved in PAF-induced eosinophil recruitment, an interleukin(IL)-5 dependent response (Whelan *et al*, 1994), are more sensitive to inhibition by systemic glucocorticoids than are the cells involved in LPS-induced neutrophil recruitment, an IL-5 independent response (Whelan *et al*, 1994). However, further studies are required to identify the target cells involved in the responses to glucocorticoids in inhibiting granulocyte accumulation in the lung.

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590P DEXAMETHASONE INHIBITS ALLERGEN-INDUCED EOSINOPHIL INFILTRATION AND ACTIVATION IN THE GUINEA-PIG LUNG

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Asthma is characterised by the accumulation of leukocytes, particularly eosinophils, in the lung. Anti-inflammatory steroids are widely used in the treatment of asthma and we have now investigated the effects of dexamethasone on antigen-induced eosinophil recruitment and activation in a model of allergic asthma in the guinea pig.

Guinea-pigs (350g) were sensitized by i.p. administration of 1ml of a suspension containing ovalbumin (10μg), Al $(OH)_3$ (10mg-^{1}) and Bordetella pertussis (1 x 10^9 organisms) on days 1, 14 and 28. On day 42 animals were challenged by inhalation of saline (sham) or 0.1% ovalbumin for 30 min. Dexamethasone (0.3, 3 or 30mgkg-^{1}) or solvent (saline) was administered (i.p.) 2h pre-, 6h post- and 24 hour post-challenge. Animals were culled 48h post-challenge and bronchoalveolar lavage (BAL) performed and blood collected for differential cell counts. BAL fluid and cells were assayed for eosinophil peroxidase (EPO) as a marker of eosinophil activation (Strath et al, 1985).

Ovalbumin challenge caused a significant recruitment of leukocytes into the BAL fluid, these were predominantly eosinophils which increased by 1220%. Dexamethasone inhibited leukocyte infiltration in a dose related manner (table 1). Ovalbumin challenge also activated eosinophils since, compared to sham, EPO in the BAL fluid increased by 228% while that present inside the eosinophil was reduced by 73%. Dexamethasone also dose relatedly inhibited eosinophil activation. Compared to control EPO levels in the BAL fluid were reduced by 8, 49 and 83% while intracellular EPO in the eosinophil was increased toward sham levels. Neither challenge nor dexamethasone had an effect on blood leukocyte counts. These data demonstrate a marked anti-inflammatory profile for dexamethasone, albeit at low potency, in the guinea pig.

TABLE 1 **Cell counts (x10^6ml-^{1}) in BAL fluid and EPO/10^6 eosinophils (ngml-^{1})**

Group	n	Eosinophils	Macrophages	Lymphocytes	Neutrophils	Total	Cellular EPO
Sham	4	0.5 ± 0.3	5.0 ± 0.7	0.8 ± 0.3	0.6 ± 0.2	6.6 ± 0.9	2778 ± 120
Control	9	6.6 ± 1.3	11.0 ± 1.3	2.9 ± 0.5	3.8 ± 0.6	24.2 ± 2.8	750 ± 80
Dexamethasone							
0.3mgkg-^{1}	9	4.7 ± 1.3	5.9 ± 0.7*	2.6 ± 0.4	1.8 ± 0.4*	15.0 ± 2.6	915 ± 92
3mgkg-^{1}	9	3.8 ± 0.6*	10.7 ± 1.9	2.1 ± 0.6	1.9 ± 0.8*	18.6 ± 3.2	1440 ± 101*
30mgkg-^{1}	9	0.5 ± 0.2***	3.7 ± 0.4***	0.5 ± 0.1**	0.3 ± 0.2**	5.1 ± 0.6***	2498 ± 122**

Data = mean ± sem; *p<0.05, **p<0.1, ***p<0.001, compared to control, ANOVA

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591P SOME BIOLOGICAL ACTIVITIES OF A NEW CORTICOSTEROID, FLUTICASONE PROPIONATE

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Asthma is now widely recognised as an inflammatory disease and recent recommendations have suggested that inhaled corticosteroids should be considered as first-line therapy (Bardin & Holgate, 1991). Lymphocytes have been implicated as one of the inflammatory cell types responsible for the chronicity of the disease (Azzawi *et al*, 1990) and inhaled corticosteroids have been shown to reduce lymphocytic infiltration into the lungs of asthmatics (Djukanovic *et al*, 1992).

Accordingly, the new corticosteroid, fluticasone propionate (FP), has been compared against beclomethasone dipropionate (BDP) and budesonide (BUD), both of which have been shown to be effective therapies in asthma. The three corticosteroids were compared in an *in vitro* human T-cell anti-proliferative assay and for their abilities to produce vasoconstriction on normal human skin. The latter has been accepted as an indication of local anti-inflammatory activity in man.

For the T-cell anti-proliferative assay, freshly isolated human lymphocytes were incubated for 48 hours in the presence of a monoclonal antibody to CD3 (anti-CD3) and varying concentrations of the corticosteroids. (^{3}H-)Thymidine was added for a further 16 hours; the cells harvested and counted. The corticosteroids produced concentration-dependent reductions in (^{3}H)-thymidine incorporation such that IC_{50}-values could be calculated. Vasoconstrictor potencies were estimated by applying four doses of each corticosteroid topically to the fore-arms of 24 subjects, occluding overnight and scoring the vasoconstriction the following day. Dose-dependent increases in vasoconstrictor scores were obtained such that relative potency estimates could be made using fluocinolone acetonide (FA=1.0) as the standard.

Table 1.	Inhibition of T-cell proliferation IC_{50} (nM)	Vasoconstrictor potency (FA=1.0)
FP	0.3 (0.2-0.4)*	7.6 (4.8-12.1)
BDP	2.0 (1.3-3.2)	4.0**
BUD	0.8 (0.5-1.3)	2.2 (1.4-3.5)

* Figures in brackets are fiducial ranges at P=0.95 **Mean of two assay results.

The results are given in Table 1. FP was respectively 6 and 3 times more potent than BDP and BUD at inhibiting human T-cell proliferation. When applied to human skin, FP was 2 to 3-fold more potent than BDP and BUD. Clinically, BDP and BUD have been shown to be equi-effective when given by inhalation to asthmatics. In this study FP was shown to be at least 2-fold more potent than both BDP and BUD and therefore might be expected to be more potent than either when administered into the lungs of asthmatics.

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592P INHALED ROLIPRAM AND ZAPRINAST INHIBIT AIRWAY MICROVASCULAR LEAKAGE PRODUCED BY INHALED PLATELET-ACTIVATING FACTOR IN THE GUINEA-PIG

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Airway microvascular leakage (AMVL) is an important component of airway inflammation and may contribute to the pathophysiology of asthma (Persson, 1986). Isoenzyme-selective inhibitors of phosphodiesterase (PDE) III, IV & V may be useful in asthma (Torphy & Undem, 1991). We have now examined the efficacy of inhaled milrinone (PDE III inhibitor), rolipram (PDE IV inhibitor), and zaprinast (PDE V inhibitor) to inhibit platelet-activating factor (PAF)-induced AMVL in guinea-pigs. The effect of inhaled salbutamol was also evaluated for comparison. Tricoloured guinea-pigs were prepared essentially as outlined by Tokuyama et al. (1991). Aerosols were generated by an ultrasonic nebulizer. Extravasated Evans blue dye content was measured in trachea (T), main bronchi (MB), and proximal (P-) and distal (D-) intrapulmonary airways. Animals were given nebulized vehicle, milrinone (10 mg/ml), rolipram (0.01, 0.1, 1, or 10 mg/ml), zaprinast (1 or 10 mg/ml), or salbutamol (0.1, 1, or 10 mg/ml) for 60 breaths. Evans blue dye (20 mg/kg, i.v.) was administered 30 min later. Nebulized PAF (0.6 mM) or the diluent of PAF was administered for 30 breaths one min later. There were no significant differences at any airway level in extravasation of dye after sham stimulation with inhaled diluent of PAF in animals pretreated with drugs or drug vehicles. Inhaled PAF increased the amount of extravasated dye compared to the control animals at all airway levels (values for dye content of T, MB, P-IPA and D-IPA were, respectively, 48±5, 49±5, 44±6, 36±5 [mean±s.e. mean] ng/mg, n=16). Rolipram, zaprinast, and salbutamol, each at 10 mg/ml, reduced extravasation of dye after PAF at all airway levels but milrinone was not effective. Values for dye content of T, MB, P-IPA and D-IPA were, respectively, 13±6, 8±3, 7±2, 9±2 ng/mg for rolipram, 19±7, 23±4, 17±5, 13±3 ng/mg for zaprinast, and 27±4, 18±3, 18±5, 16±6 ng/mg for salbutamol (n=5 for each group; $P<0.05$ vs. PAF). Rolipram (1 mg/ml) was also effective. These results confirm and extend previous reports on the anti-exudative effect of systemic or intratracheal administration of PDE IV & V inhibitors (Raeburn & Karlsson, 1992; Ortiz et al., 1992) and indicate that this pharmacological approach is of potential therapeutic value in asthma.

Rolipram and milrinone (Almirall, Spain) and zaprinast (Rhône-Poulenc Rorer, U.K.) were gifts. Funded in part by FAR91-875, SAF 93-132 (M° Industria, Spain).

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593P THE EFFECT OF OXYGEN TENSION ON RESPONSES EVOKED BY METHACHOLINE AND BRONCHODILATORS IN ISOLATED BOVINE BRONCHIAL RINGS

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Most studies of isolated tissue function have been conducted in 95% O_2, which may not give an indication of the response at physiological O_2 tensions. We examined, in bovine bronchi, the effect of altering O_2 tension on the actions of the constrictor methacholine (MCh) and the bronchodilators; salbutamol, atrial natriuretic peptide (ANP), isosorbide dinitrate (ISDN) and sodium nitroprusside (SNP)

Contractions of rings of bovine bronchi (3rd-5th order) were measured isometrically. Tissues were preconstricted with MCh ($3x10^{-6}$M) and the ability of the bronchodilators to evoke relaxation was tested in 95, 20 and 4% O_2. When ANP was used, phosphoramidon (Ph, $3.7 x10^{-5}$M) was present to prevent its rapid breakdown. Results are shown in the table below (n=6 in each case).

Drug	95% O_2 (% inhib.)	20% O_2 (% inhib.)	4% O_2 (% inhib.)	***p<0.001 c/f 95%, †p<0.05 c/f 20%, ††† p<0.001 c/f 20%
Salbutamol (10^{-6}M)	72± 14	14±7 ***	30±14***†	
ANP (10^{-6}M)	39±12	14 ±3***	40±13†††	
ISDN ($4.2x10^{-5}$M)	71±13	83±8	82±13	
SNP (10^{-6}M)	38± 11	34±8	45±13	

In a second series of experiments, cumulative concentration-response curves to MCh (CRCs, 10^{-9}-$3x10^{-4}$M) were constructed in the presence and absence of each of the above bronchodilators. O_2 tensions of 4 and 20% each significantly ($p<0.001$) enhanced contractions to MCh alone compared to those in 95%, with the order of enhancement being: 20% > 4%.

Salbutamol in 95% oxygen evoked a significant ($p<0.001$) rightward shift of the MCh response but had no effect in 4 and 20%. In 95% oxygen, ANP caused a significant ($p<0.001$) rightward shift of the CRCs , whilst in 20% ANP was ineffectual and at 4%, it evoked a leftward ($p<0.001$) shift. The mechanism behind this is as yet unknown, however this response could not be attributed to Ph, as alone, Ph evoked no changes in the MCh curves at any O_2 tension used. ISDN caused an increasing rightward shift ($p<0.05$, $p<0.01$ and $p<0.001$ in 95, 20 and 4% oxygen, respectively) in decreasing oxygen tensions. SNP produced no effect at the O_2 tensions used.

Altering O_2 tension markedly altered contractions to MCh and affected the ability of some, but not all, of these dilators to reverse or protect against subsequent MCh challenge. The differences between ISDN and SNP, both in potency and sensitivity to O_2 tension, are surprising given that both agents are proposed to act via soluble guanylate cyclase activation, while ANP, which is thought to act via particulate guanylate cyclase activation showed a differing pattern again.

In conclusion, the effectiveness of methacholine and each of the bronchodilators used was inherently dependent on the surrounding oxygen tension. This may have clinical ramifications for patients suffering from respiratory disorders, such as asthma.

This work was supported by the Wellcome Trust, the Chest Heart and Stroke Assoc. and the National Asthma Campaign.

594P EFFECT OF CHARYBDOTOXIN ON INHIBITION OF HISTAMINE-STIMULATED INOSITOL PHOSPHOLIPID HYDROLYSIS BY ISOPRENALINE, SALBUTAMOL AND SALMETEROL IN BOVINE TRACHEAL SMOOTH MUSCLE

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In common with other β_2-adrenoceptor agonists, salmeterol (SALM) stimulates cyclic AMP (cAMP) accumulation in bovine tracheal smooth muscle (BTSM; Ellis *et al.*, 1994). One consequence of increasing cAMP accumulation is inhibition of histamine (HIST)-stimulated [^{3}H]inositol phosphate ([^{3}H]InsP) accumulation, perhaps at least in part as a consequence of activation of large conductance Ca^{2+}-dependent K^+-channels (K_{Ca}) in airway smooth muscle (see Challiss *et al.*, 1993). Recently, the actions of SALM have been reported not to involve K_{Ca} channels (Cook *et al.*, 1993). In the present study, we have compared the ability of SALM to cause inhibition of HIST-stimulated [^{3}H]InsP accumulation in BTSM compared to the actions of salbutamol (SALB) and isoprenaline (ISO). In addition, the effects of pre-application of the K_{Ca} blocker, charybdotoxin (ChTX) have been studied.

BTSM slices (300x300 μm) were maintained in culture medium in the presence of 0.8 μCi [^{3}H]inositol/ml for 12-24 h. Slices were washed into oxygenated Krebs-Henseleit buffer (KHB). 75 μl packed slices were dispensed into 400 μl KHB containing 0.8 μCi [^{3}H]inositol/ml and 5 mM LiCl. Where indicated, incubations were carried out in the presence of ChTX (3, 30 or 300 nM). SALM (0.1-300 nM), SALB (1nM-10 μM) or ISO (10 μM) were added 20 min prior to the addition of HIST (100 μM). Incubations were then terminated 30 min after HIST addition and [^{3}H]InsP accumulation was determined as described previously (Challiss *et al.*, 1992).

HIST stimulated an increase in [^{3}H]InsP accumulation from a basal level of 1700±145 to 10929±1286 d.p.m./mg protein in 30 min. All three β-agonists tested caused similar maximal (SALM (100 nM) 63.7±3.2; SALB (1 μM) 60.2±9.9 and ISO (10 μM) 71.5±5.9%)inhibitions, with SALM more potently inhibiting HIST-stimulated [^{3}H]InsP accumulation compared to SALB (IC_{50}s (geometric mean [range]) 1.4 [0.3-6.3] and 13.8 [7.0-27.4] nM respectively). ChTX *per se* (3-300 nM) did not affect basal values, but increased HIST-stimulated [^{3}H]InsP levels, this enhancement being maximal at 30 nM ChTX (17365±3220 d.p.m./mg protein). In the presence of 30 nM ChTX the inhibition of [^{3}H]InsP accumulation by 100 nM SALM was attenuated (from 59.7±3.5 to 26.0±8.6% (expressed as a % of [^{3}H]InsP accumulation in the presence of HIST and HIST+ChTX respectively). However, it was only in the presence of a higher concentration of ChTX (300 nM) that the inhibition of the HIST-stimulated response by 1 μM SALB (from 55.5±3.2 to 22.9±10.2%) or 10 μM ISO (from 70.7±3.6 to 42.7±8.2%) was significantly reduced.

These studies demonstrate that the maximal inhibitions of HIST-stimulated [^{3}H]InsP accumulation caused by SALB, ISO and SALM are similar, with SALM exhibiting a greater potency compared to SALB. We also find that SALM-induced inhibition of [^{3}H]InsP accumulation is more sensitive to blockade of K_{Ca} by ChTX than either SALB or ISOP. The effectiveness of ChTX against the β-agonists indicates either that the toxin may be acting as a functional antagonist (by promoting Ca^{2+}-influx) or that it directly prevents β-agonist-induced opening of K_{Ca}. Furthermore, different abilities of β-adrenoceptor agonists to affect phosphoinositide turnover in the presence of ChTX may reflect efficacy differences between these agents.

Financial support was provided by Glaxo Group Research.

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595P POTENCY, SELECTIVITY AND DURATION OF ACTION OF ARALKYLOXYALKYL ANALOGUES OF β- ADRENOCEPTOR BLOCKING DRUGS: PARALLELS WITH SALMETEROL

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Previous studies have shown that N-substitution with aralkyloxyalkyl groups in salbutamol and clenbuterol yields the potent, highly selective and long-acting β_2-adrenoceptor agonists, salmeterol (Bradshaw *et al.*, 1987) and picumeterol (GR114297A, Nials *et al.*, 1993). Although the mechanism of the extended duration of action of these compounds is not fully established, it is believed to result from an interaction of the side chain in the molecule with an *exo-site* adjacent to the active site of the β_2-adrenoceptor. We have now studied the effect of introducing similar aralkyloxyalkyl N-substituents into the structures of two non-selective β_1/β_2-adrenoceptor blocking drugs, propranolol (PROP) and sotalol (SOT), to yield hexyloxyethylpyridyl-PROP (GR81706) and hexyloxyethylphenyl-SOT (GR76092).

β_2-adrenoceptor antagonist potency was evaluated against salbutamol or isoprenaline-induced relaxant activity in superfused, electrically-stimulated guinea-pig trachea (ESGPT, Coleman & Nials, 1989). β_1-adrenoceptor antagonist activity was assessed against isoprenaline-induced inotropic responses in electrically-driven rat left atria (RA, Juberg *et al.*, 1985).

These structural modifications resulted in apparent 8 and 2-fold reductions in the β_2-adrenoceptor blocking potencies of PROP and SOT respectively on guinea-pig trachea (pA_2 [95% c.l.]: PROP 9.3 [8.1-10.2], GR81706 8.4 [7.3-9.6]; SOT 7.3 [6.9-7.8], GR76092 7.0 [5.9-8.1]), and 200- and 10-fold reductions in β_1 adrenoceptors blocking potencies on RA (pA_2s: PROP 9.2 [8.3-10.2], GR81706 6.9 [6.1-8.0], SOT 6.9 [6.3-7.4], GR76092 5.9 [4.3-7.4]). In addition, in each case, the aralkyloxyalkyl substitution markedly extended the duration of action of the antagonist against a continuous infusion of isoprenaline in the ESGPT. The times, after stopping the antagonist infusion, for the isoprenaline-induced inhibition to reassert by 50% (Rta_{50} [95% c.l.] were : PROP 165 [78-348] min, GR81706 > 420 min; SOT 28 [17-41] min, GR76092 > 420min). The aralkyloxyalkyl chain therefore causes an apparent increase in β_2-adrenoceptor selectivity, and markedly extends the duration of β-adrenoceptor activity in both agonists and antagonists suggesting that it is specifically interacting with the receptor protein. The availability of long-acting β-adrenoceptor blocking drugs may help in the elucidation of the mechanism of the prolonged duration of action of salmeterol and related compounds.

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596P DEVELOPMENT OF A METHOD TO ASSAY SALBUTAMOL DELIVERED FROM COMMERCIAL FORMULATIONS BY ITS BRONCHODILATOR EFFECTS IN NORMAL HUMAN SUBJECTS

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The delivery of drugs directly into the respiratory tract is the method of choice for treatment or prophylaxis of asthma. The imposition of a steady state of bronchoconstriction by inhalation of loading and maintenance doses of methacholine allows bronchodilator drug evaluation in normal subjects (Foster *et al.*, 1991). An ideal assay for inhaled bronchodilator drugs would be a parallel line multi-point one bracketing the midpoint. However, the problem with commercial formulations is their high unit dose; this almost forces use of a one-point assay, so the relative potency must be calculated by an indirect method. The log dose response curve (LDRC) to inhaled nebulized salbutamol was used to provide a reference for each formulation in calculating its relative potency.

Six normal subjects were used; all experiments were performed 3 times and all measurements of specific airway conductance (sG_{aw}) were means of 5 estimates. After measurement of baseline sG_{aw} and establishment of the constricted steady state, Aerolin Autohaler (breath-actuated MDI) 100 µg, Rotacaps (dry powder inhaler) 200 µg and Nebulized (Respirator solution) 140 µg salbutamol were compared in a randomized cross-over design. The sG_{aw} was measured frequently between 5 and 19 min and then at 20 min intervals to 100 min after dosing. The peak amplitude of effect, interpolated in the amplitude-bracketing segment of the subject's mean nebulized salbutamol LDRC, provided the relative potency as the $\log_e$ dose ratio. The rate of offset of bronchodilator effect was described by the linear regression slope. The results were compared using paired *t*-tests.

There were no significant differences over the 3 protocols between baseline ($P \approx 0.29$-0.82) or constricted steady states ($P \approx 0.29$-0.5). A major increase in $\log_{10} sG_{aw}$ was seen within 9 min of inhalation of each formulation, that stayed at that peak until about 19 min. Rotacaps showed a larger relative potency (mean±sem -0.22±0.34) than Aerolin Autohaler (-1.23±0.28; $P \approx 0.03$). There was no significant difference in relative potency between Aerolin Autohaler and Nebulized (-0.92±0.20) ($P \approx 0.42$) or between Rotacaps and Nebulized salbutamol formulations ($P \approx 0.15$). Rotacaps showed a larger rate of offset (-0.0021±0.0003 $\log_{10}$ (s $cmH_2O)^{-1}$ min^{-1}) than Nebulized (-0.0013±0.0003; $P<0.01$). There was no significant difference in rate of offset between Aerolin Autohaler (-0.0019 ±0.0002) and Rotacaps ($P \approx 0.6$) or between Aerolin Autohaler and Nebulized salbutamol formulations ($P \approx 0.2$).

These results suggested that 1) salbutamol formulations produced a peak of bronchodilatation 9-19 min after dosing; 2) Rotacaps was more potent than Aerolin Autohaler but equipotent with Nebulized: Aerolin Autohaler was equipotent with Nebulized salbutamol; 3) the effect of salbutamol delivered from Rotacaps (200 µg) wore off more rapidly than that from Nebulized (140 µg) but not than that from Aerolin Autohaler (100 µg): Aerolin Autohaler (100 µg) and Nebulized (140 µg) salbutamol showed similar mean rates of offset; 4) this one point assay method provides a satisfactory bioassay of commercial formulations of bronchodilator drugs.

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597P COMPARISON OF THE EFFECTS OF ISOPRENALINE AND BRL 37344 IN TISSUES OF THE RAT ALIMENTARY TRACT

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Atypical β-adrenoceptors (β_3-adrenoceptors) have been identified in tissues of the gastrointestinal tract of several species e.g guinea-pig ileum, guinea pig and rat gastric fundus, rat and rabbit jejunum, rat colon (see McLaughlin & MacDonald, 1991 for references). It is not known whether the atypical β-adrenoceptors are distributed homogeneously throughout the tract of any one species or whether there are regional differences which might be related to function. The present study compared the effects of isoprenaline, the prototype β-adrenoceptor agonist with BRL 37344, a selective β_3-adrenoceptor agonist in the presence of propranolol and prazosin to block classical β-adrenoceptors and α_1-adrenoceptors respectively. Differences in the potency ratios of BRL 37344 and isoprenaline at atypical β-adrenoceptors may reflect tissue factors, such as efficiency of receptor coupling (Kenakin, 1987) or differences in the receptors at different sites.

Longitudinal preparations of rat gastric fundus (prepared as strips), oesophagus muscularis mucosae, proximal small intestine, jejunum, ileum, proximal and distal colon (all prepared as segments) were suspended in Krebs solution at 37°C for isometric recording. The Krebs solution contained EDTA (30 µM), ascorbic acid (30 µM), propranolol (1 µM) and prazosin (0.1 µM) and was gassed with 95/5% O_2/CO_2. After an initial equilibration period, reproducible contractions to a submaximal concentration of methacholine (1 µM) were obtained before carrying out concentration response curves (CRCs) to isoprenaline and BRL 37344 in paired tissues. CRCs were constructed non-cumulatively, except in the case of oesophagus muscularis mucosae where cumulative CRCs were carried out due to the length of time the preparation took to contract on addition of methacholine and to relax on washing.

Both isoprenaline and BRL 37344 produced a concentration-dependent inhibition of tension generated by methacholine with the following order of sensitivity: distal colon (most sensitive)> oesophagus > proximal colon > ileum > fundus > proximal small intestine = jejunum (least sensitive). In the oesophagus and ileum isoprenaline and BRL 37344 were approximately equipotent and a maximum inhibition of 70 - 100 % of methacholine tension was obtained. In the proximal small intestine and jejunum isoprenaline and BRL 37344 were approximately equipotent but only 40 - 60 % inhibition of methacholine tension was observed. In the gastric fundus BRL 37344 was approximately half as potent as isoprenaline and both agonists produced 70 - 90 % inhibition of methacholine tone. In the proximal and distal colon BRL 37344 was slightly more potent than isoprenaline. The agonists produced greater inhibition in distal (80 - 100 %) compared to proximal (70 - 80 %) colon.

In conclusion, differences in the relative potencies of isoprenaline and BRL 37344 at atypical β-adrenoceptors in different parts of the gastrointestinal tract were found. Differences in the potency ratios and in the maximum responses obtained possibly reflect differences in efficiency of receptor coupling.

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598P INVOLVEMENT OF HETEROTRIMERIC G-PROTEINS IN THE CONTROL OF EXOCYTOTIC MEMBRANE FUSION IN THE EXOCRINE PANCREAS

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GTP-binding proteins are known to be involved in the control of membrane fusion during regulated exocytosis (Gomperts, 1990). Recently, evidence has emerged that the proteins involved are heterotrimeric G-proteins similar to those that couple cell-surface receptors to second messenger systems (Vitale *et al.*, 1993). We have shown previously (Edwardson *et al.*, 1990) that GTPγS stimulates exocytosis in the exocrine pancreas. Here we have characterised the G-proteins that mediate this effect.

Exocytotic membrane fusion was studied both in permeabilized pancreatic acini and in an *in vitro* system based on isolated zymogen granules and plasma membranes. Acini were prepared by collagenase digestion of rat pancreas (Edwardson *et al.*, 1990), and permeabilized using streptolysin O (0.2 U ml^{-1}). Exocytosis was triggered by addition of 10 μM free Ca^{2+}, and assayed through the release of amylase over a 45-min incubation at 37°C. Release was expressed as a percentage of total amylase content. Zymogen granules and plasma membranes were isolated as described (MacLean & Edwardson, 1992). Fusion between granules and plasma membranes was assayed through the dilution-dependent de-quenching of the fluorescence of octadecylrhodamine B-chloride, which had been loaded into the granule membranes (MacLean & Edwardson, 1992).

AlF_{3-5} (50 μM $AlCl_3$ + 10 mM KF) increased amylase release in permeabilized acini from 4.4±0.1% (n=6) to 7.0±0.1% (n=10; P<0.001). The Al^{3+}-chelator deferoxamine (500 μM) reduced amylase release in the presence of AlF_{3-5} to 3.6±0.1% (n=6; P<0.001). In acini pre-treated with cholera toxin, Ca^{2+}-stimulated exocytosis was increased from 1.6±0.1% (n=12) to 2.5±0.1% (n=12; P<0.001), and GTPγS caused only a small additional enhancement, to 3.1±0.2% (n=12; P<0.01), a level not significantly different from that in control acini (2.9±0.2%, n=12). Similar effects of both AlF_{3-5} and cholera toxin were also seen in the *in vitro* system. Exocytotic membrane fusion, both in permeabilized acini and *in vitro*, was inhibited by benzalkonium chloride, an activator of G_o (Higashijima *et al.*, 1990), with an EC_{50} of approximately 5 μg ml^{-1}.

These results indicate that exocytotic membrane fusion in the exocrine pancreas may be stimulated via a heterotrimeric G-protein of the G_s-type and inhibited via a G_o-type G-protein. The predominant effect of GTPγS and AlF_{3-5} is stimulatory. The location and physiological role of these G-proteins are presently under investigation.

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599P ACTIVATION OF ADIPOCYTE HORMONE-SENSITIVE LIPASE IN MICE BY β_3 ADRENOCEPTOR AGONISTS

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We have previously shown that selective β_3 adrenoceptor agonists stimulate brown adipose tissue lipogenesis *in vivo* (Al Qatari & Taberner, 1992a,1992b). *In vitro* studies in adipocytes by Hollenga et al. (1991), comparing cAMP production and lipolysis, have suggested that both β_1 and β_3 receptor activation may be involved. More recently, it has been reported that β_3-adrenoceptor-induced lipolysis can be correlated with the activation of cAMP-dependent protein kinase in white adipocytes *in vitro* (Murphy et al., 1993). Since adrenoceptor regulation of lipolysis is exerted via hormone sensitive lipase (HSL) we have measured the effects of adrenoceptor agonists *in vivo* on HSL activity in brown (BAT) and white (WAT) adipose tissue and cAMP production, following the same dosing schedules used previously.

HSL was assayed using ^3H triolein as substrate (Huttunen et al., 1970). cAMP accumulation was measured by a saturation binding assay (Shepherd et al., 1977). Groups of 6-7 male CBA mice were treated with drugs (i.p.) or saline as control 1 hr prior to the assays. Propranolol (10 $mg.kg^{-1}$) or saline as control was administered 30 min prior to the agonist. Differences between groups (means ± sem) were analysed by Students t-test.

HSL activity in BAT was significantly increased ($p < 0.01$) from 5.03 ± 0.32 to 8.0 ± 0.71 nmol $FFA.min^{-1}.mg\ protein^{-1}$ by noradrenaline (NA, 0.6 $mg.kg^{-1}$) and to 10.54 ± 0.78 ($p < 0.001$) by the β_3 agonist BRL 35135A (5 $mg.kg^{-1}$). Propranolol, at a dose which did not in itself alter HSL activity, blocked the activation by NA and BRL 35135A. The other β_3 agonists tested (SR 58611A: 2$mg.kg^{-1}$ and ICI D7114: 10 $mg.kg^{-1}$) produced similar levels of activation. Similar effects were observed on cAMP production although the increases were not as great; control: 1520 ± 102 pmol. $mg\ protein^{-1}$ increased to 2120 ± 103 after NA and 1817 ± 27 after BRL 35135A. In WAT, in contrast, the same dose of BRL35135A and the other selective β_3 agonists had no effect on HSL activity (control: 5.54 ± 0.37; 5.84 ± 0.35 after BRL 35135A) or cAMP production (control: 2261 ± 181; 2257 ± 263 after BRL 35135A), although NA was still effective (HSL increased to 8.57 ± 0.15 ($p < 0.01$); cAMP increased to 2510 ± 23). The agonist doses were those which had previously been shown from concentration-response curves to be maximal for stimulating *in vivo* lipogenesis (Al Qatari, 1993).

It would appear that β_3 agonists, unlike NA, can be selective towards HSL activation of BAT rather than WAT lipolysis *in vivo* and that cAMP accumulation and HSL activity in BAT are linked to β_3 adrenoceptor activation.

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600P OXIDATION OF LOW-DENSITY LIPOPROTEINS IN THE PRESENCE OF BOVINE AORTIC ENDOTHELIAL CELLS

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We have shown that exposure of bovine aortic endothelial cells (BAEC) to low density lipoproteins (LDL) modifies intracellular processes in a proatherogenic manner (Thorin et al, 1994; Hamilton et al, 1994). These changes in cellular biochemistry may be consequent on LDL oxidation. To explore this hypothesis we studied the oxidation of LDL in the presence of BAEC.

LDL was prepared from human plasma by sequential ultracentrifugation. LDL from each sample was oxidised with Cu^{2+} (4 μM for 24 hrs at room temperature) to give maximum oxidation and by incubation over BAEC. Cells prepared by enzymatic digestion were plated into 1 cm diameter multiwells at 10^5 cells/well and grown to confluence. Growth medium was replaced by M199 without phenol for 24 hrs and finally by M199 ± LDL 20 μg/ml for 18 hrs. Additional wells contained M199 ± LDL but no cells. In further studies the antioxidant N acetylcysteine (NAC) 10 μM was added to some wells. Oxidised LDL formed during incubation with Cu^{2+} or endothelial cells was quantified by assay for thiobarbituric acid reactive substances (TBARS) and expressed as nmoles malondialdehyde (MDA)/mgprotein (Yagi 1987). Results are expressed as mean ± SD with n = 7-12.

Cu^{2+} oxidation of LDL resulted in TBARS of 9.6 ± 0.3 nmoles MDA/mgprotein. TBARS were undetectable in LDL and minimal after incubation of LDL in the absence of cells (1.1 ± 0.4 nmoles MDA/mgprotein. In the presence of BAEC TBARS equivalent to 3.9 ± 0.7 nmoles MDA/mgprotein were detected. Addition of NAC reduced this to 2.3 nmoles ± 0.4 MDA/mgprotein.
We have previously shown that native LDL has up to 50% the potency of oxidised LDL in modifying phosphatidylinositol metabolism, prostacyclin production, and intracellular calcium concentrations and that these effects are attenuated by antioxidants. The results presented here are consistent with the hypothesis that many of the effects of native LDL on BAEC are related to oxidation of LDL during incubation.

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601P CYTOCHROME P450-DEPENDENT MONOOXYGENASE: A MAJOR ROLE IN THE VASOCONSTRICTOR RESPONSES OF RAT MESENTERIC ARTERIES?

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We have previously demonstrated the presence of both cyclooxygenase and lipoxygenase derived vasoconstrictors in rat small mesenteric arteries (Wu et al, 1994). In this study, in an attempt to further define the lipoxygenase product, we have examined the effects of selective inhibition of 5- (BWA4C), 12- (baicalein) and 15-lipoxygenase (RG6866) and cytochrome P450-dependent monooxygenase (SKF525A), on the contractile properties of small mesenteric arteries. Third branch mesenteric arteries (n=82, mean internal diameter 166±6 μm) from male Wistar rats (weight 280-400 g) were mounted in a myograph. Concentration-response curves were constructed in groups of vessels (6-8 vessels/group) for noradrenaline (0.01-10 μM), potassium chloride (12-125 mM), acetylcholine (0.01-100 μM) and endothelin-1 (0.01 nM-1 μM), alone and in the presence of one of BWA4C (1 μM), baicalein (1 μM), RG6866 (1 μM), SKF525A (50 μM) and SKF525A (50 μM) with indomethacin (14 μM). A further series of vessels were perfused with 0.3% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), for 3 minutes, to effect endothelium removal. Concentration-response curves were constructed, in these vessels, as outlined above alone and in the presence of SKF525A.

Inhibition of 5- (BWA4C), 12- (baicalein) or 15-lipoxygenase (RG6866) did not affect either the EC_{50} or maximum response obtained to acetylcholine, noradrenaline or potassium chloride. Inhibition of cytochrome P450-dependent monooxygenase with SKF525A attenuated the contractile response to noradrenaline (EC_{50} 5.89±0.01 versus 5.71±0.01 with SKF525A, p<0.02; maximum response 5.30±0.72 mN/mm versus 3.88±0.80 mN/mm with SKF525A, p<0.005) potassium chloride (maximum response 4.77±0.40 mN/mm versus 0.27±0.04 mN/mm with SKF525A, p<0.0001) and endothelin-1 (maximum response 85.3±2.7% versus 53.9±4.2%, p<0.0001). SKF525A also attenuated acetylcholine-induced relaxation (EC_{50} 6.94±0.10 versus 5.88±0.07 with SKF525A, p<0.0001; maximum relaxation 95.1±1.9% versus 76.0±2.8% with SKF525A, p<0.0001). The inhibitory affect of SKF525A was additional to that of indomethacin (ind) for contractions induced by both noradrenaline (EC_{50} 5.82±0.05 versus 5.50±0.05 with SKF525A and ind, p<0.01; maximum response 6.61±0.47 mN/mm versus 2.74±0.38 mN/mm with SKF525A and ind, p<0.0001) and potassium chloride (maximum response 5.84±0.54 mN/mm versus 0.10±0.05 mN/mm with SKF, p<0.0001). Endothelial removal with CHAPS did not attenuate the inhibitory action of SKF525A on contractions to noradrenaline (EC_{50} 6.1±0.12 versus 5.42±0.03 with SKF525A, p<0.01; maximum response 3.20±0.52 mN/mm versus 1.53±0.23 mN/mm with SKF525A, p<0.001) and potassium chloride (maximum response 2.44±0.39 mN/mm versus 0.05±0.03 mN/mm, p<0.001).

These data suggest a role for vascular smooth muscle cytochrome P450 dependent monooxygenases in the control of vascular tone of small mesenteric arteries from Wistar rats.

Wu, X.C., Richards, N.T., Michael, J., Johns, E. (1994) Br. J. Pharmac in press

602P THE TOXICITY OF ARTEMISININ, ITS DERIVATIVES AND METABOLITES IN Nb2a NEUROBLASTOMA AND C6 GLIOMA CELLS

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Artemisinin and its derivatives are sesquiterpene lactones with an unusual endoperoxide linkage, which is thought to be responsible for their antimalarial activity. They are highly effective against the erythrocytic stages of *P. falciparum in vitro* and *in vivo* (Klayman 1985). An *in vivo* study using dogs and rats injected i.m. with artemether and arteether (Brewer *et al.*, 1993) revealed a dose-dependent neurotoxic effect associated with movement disturbances and spasticity. In this study, the effects of artemisinin, its derivatives and metabolites on Nb2a and C6 cell proliferation and Nb2a cell neurite outgrowth were examined. Desoxyartemisinin and desoxyarteether are metabolites of artemisinin and arteether that lack the endoperoxide linkage and were used to determine the importance of this functional group to toxicity.

Cells were maintained in DMEM medium with 5-10% foetal calf serum until confluent. In the cell proliferation experiments, Nb2a cells were incubated with artemisinin, its derivatives or metabolites for 48h; C6 cells were incubated with artemisinin for 5 days. The concentration of the compounds used was 0.1 to 100μM. 0.2μCi of ^{3}H-thymidine was added per 1200 cells and, after cell harvesting, the incorporation of ^{3}H-thymidine was measured by liquid scintillation counting. In the neurite outgrowth experiments, serum was withdrawn from the Nb2a cells and 500μM dibutyryl cyclic AMP and 0.1 to 100μM of the compound added. The cells were then incubated for 24h, fixed and stained with 4% paraformaldehyde and Coomassie blue cell stain. The cells were examined by light microscopy and the number of neurites and extensions on the cells counted. Statistical significance was determined by paired and unpaired t-tests. A p-value of < 0.05 was considered significant.

All compounds except desoxyartemisinin significantly inhibited C6 and Nb2a cell proliferation in a dose-dependent manner. Desoxyartemisinin had no significant effect on proliferation at any concentration. None of the compounds significantly inhibited Nb2a cell proliferation at 0.1μM except artemisinin. Rank order of toxicity: IC_{50} values, (n=4-6), expressed as geometric mean (95% confidence limits) in μM: artemisinin 0.3 (0.1-0.8) > dihydroartemisinin 1.6 (-1.7-7.1) > arteether 4.3 (-3-15.7) > artemether 13.6 (0.7-29.9) > sodium artelinate 44.2 (17.9-76.1) > desoxyarteether ≈ desoxyartemisinin >100. Rank order of toxicity in the C6 cells was (all n=4) : arteether 0.3 (0.3-0.4) > artemether 0.7 (-0.3-2.2) > dihydroartemisinin 6.4 (-28.8-85.1) > desoxyartemisinin >100. Treatment of differentiating Nb2a cells with artemether, arteether and dihydroartemisinin led to a significant dose-related decrease in the number of cells with neurites and/or extensions at concentrations of the compounds as low as 0.1μM, which suggested a specific effect on the mechanisms of neurite outgrowth at low concentrations. Our data show that artemisinin and its derivatives are toxic to Nb2a and C6 cell cultures and suggest that the endoperoxide linkage is necessary for most of this toxicity.

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603P IS Ca^{2+} CHANNEL OPENING RESPONSIBLE FOR THE STIMULATION OF INS(1,4,5)P_3 FORMATION BY μ-OPIOIDS IN SH-SY5Y CELLS?

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We have recently reported that mu, but not delta, opioids transiently (peaks at 15s) stimulate Ins(1,4,5)P_3 formation in SH-SY5Y cells, and that this is extracellular Ca^{2+} dependent (Smart et al., 1994). One possible mechanism for this effect is that mu-opioids could open a Ca^{2+} channel, as seen with kappa opioids in astrocytes (Eriksson et al., 1993), thus allowing Ca^{2+} influx to activate phospholipase C (PLC) (Park et al., 1992). To test this hypothesis we have further characterized the Ca^{2+} dependency of this response. Whole cell suspensions (final volume 0.3ml) were preincubated at 37°C for 15min in Ca^{2+}-containing or Ca^{2+}-free Krebs buffer, in the presence or absence of nifedipine (1nM to 100μM). Some of the cell suspensions were then incubated with fentanyl (0.1μM) in the presence or absence of nifedipine (1nM to 100μM) for 15s. In some experiments Ca^{2+} was replaced at 1-11min after the fentanyl challenge. Other cell suspensions were incubated with K^+ (20 to 100mM) for 0-300s. Ins(1,4,5)P_3 was measured using a specific radioreceptor mass assay (Challiss et al. 1988). In the absence of extracellular Ca^{2+} fentanyl failed to stimulate Ins(1,4,5)P_3 formation.Ca^{2+} replacement restored the stimulation of Ins(1,4,5)P_3 formation by fentanyl, but there was a time-dependent reduction in the peak Ins(1,4,5)P_3 response to Ca^{2+} replacement (Table 1). Furthermore, nifedipine, the L-type Ca^{2+} channel antagonist, inhibited fentanyl-stimulated Ins(1,4,5)P_3 formation, with an IC_{50} of 60.3 ± 1.1nM (n=4). Further conformation that Ca^{2+} channel opening activated PLC is that K^+ evoked Ca^{2+} entry also stimulated Ins(1,4,5)P_3 formation, with a peak (of 36.4 ± 3.2 pmol/mg protein) at 30s, and an EC_{50} of 42.7 ± 1.0mM (n=5). These data indicate that Ca^{2+} entry can activate PLC, and that this mechanism probably accounts for the stimulation of Ins(1,4,5)P_3 formation by mu-opioids, in SH-SY5Y cells.

Table 1. The time-dependency of the effects of Ca^{2+} replacement on fentanyl-stimulated Ins(1,4,5)P_3 formation in SH-SY5Y cells.

Time of Ca^{2+} replacement after fentanyl (min)	Peak Ins(1,4,5)P_3 (pmol/mg protein)
Basal	9.15 ± 0.39
1	20.07 ± 0.61*
2	14.74 ± 1.15*
4	10.22 ± 0.91
6	11.51 ± 0.44
11	11.13 ± 0.54

(mean + SEM, where n=5. * denotes $p<0.05$ (t-test) increased compared to basal)

Financial support by Leicestershire Health Authority and the Wellcome Trust is gratefully acknowledged.

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604P THE EFFECT OF THE OPIOID ANTAGONIST, NALOXONE, ON BACLOFEN-INDUCED ANTINOCICEPTION IN MALE AND FEMALE MICE

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Baclofen, a $GABA_B$ agonist is antinociceptive in a number of animal models (Aley & Kulkarni, 1991). There is controversy as to whether there is interaction between opioid and baclofen induced antinociception (Bartolini *et al*, 1981; Sawynok, 1987). This may be due to the diverse criteria used to define antinociception, and the lack of clarification of toxic versus analgesic doses. Furthurmore, there are sometimes differences in the manner in which male and female animals respond to some drugs (Conney, 1967), so it is possible that male and female mice respond differently to baclofen. The present work was undertaken to determine whether there is a sex difference in the analgesic effect of baclofen and to investigate the effect of naloxone, an opioid antagonist on baclofen induced antinociception in male and female mice.

Groups of 10 male or 10 female CFLP mice (wt 20-25g) were used in all experiments. In the first experiment. the antinociceptive effects of baclofen and morphine in male and female mice were investigated. The latency of the mice to respond to the hot plate test (cut off time 15 s) at 55°C following the s.c. administration of baclofen or morphine was measured. In the second experiment, the effect of naloxone on baclofen and morphine induced antinociception in male and female mice was investigated. The mice were administered with naloxone s.c. 20 min prior to the administration of baclofen or morphine. In both experiments,the latency of the mice to respond to the hot plate prior to, and 15, 30, 45 and 60 min following the administration of baclofen or morphine was measured. Responses were expressed as % antinociception relative to mean group response at time 0. Results were analysed by analysis of variance and *post-hoc* Dunnett's t-test.

Both baclofen and morphine produced dose dependent antinociception. At 30 min, at which the peak antinociceptive effect of morphine occurred, morphine (8.0 mg kg^{-1}) produced antinociception in male (37.9 ± 9.1 % antinociception, $P<0.01$) and female mice (57.8 ± 9.5 % antinociception $P<0.01$). At 45 min, the peak antinociceptive effect of baclofen, baclofen (4.0 mg kg^{-1}) produced antinociception in male (45.2 ± 9.6 % antinociception, $P<0.01$) and female mice (43.7 ± 11.9 % antinociception, $P<0.05$). Naloxone dose dependently reduced morphine and baclofen induced antinociception in male and female mice. Naloxone (0.5 mg kg^{-1}) significantly reduced morphine (8.0 mg kg^{-1}) induced antinociception in both male mice (47.9 ± 10.0 to 3.9 ± 2.1 % antinociception, $P<0.01$) and in female mice (43.1 ± 11.8 to 9.3 ± 2.6 % antinociception, $P<0.05$). However, a higher dose of naloxone (4.0 mg kg^{-1}) was required to inhibit the antinociceptive effect of baclofen in female mice (58.7 ± 8.0 to 33.17 ± 10.29 % antinociception, $P<0.05$), although naloxone (2.0 mg kg^{-1}) had virtually abolished the baclofen induced antinociception in male mice (35.9 ± 6.55 to 0.4 ± 1.7 % antinociception, $P<0.01$).

These results show that male and female mice are equally sensitive to the effects of baclofen. Naloxone inhibits baclofen induced antinociception, which suggests that there is interaction between opioid and baclofen induced analgesia. However there appears to be a sex difference as baclofen treated male mice are more sensitive to naloxone than baclofen treated female mice in the hot plate test.

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605P THE INVOLVEMENT OF BRADYKININ B_1 AND B_2 RECEPTOR MECHANISMS IN CYTOKINE-INDUCED MECHANICAL HYPERALGESIA IN THE RAT

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Previously we have shown that Freund's complete adjuvant and interleukin-1ß (IL-1ß) can induce a bradykinin B1-receptor mediated hyperalgesia in the rat (Davis and Perkins, 1992, 1993). We now report on the involvement of bradykinin B1 and B2 receptors in the induction and maintenance of mechanical hyperalgesia induced by the cytokines IL-1ß, -2, -6 and -8 (specific activities of $1x10^8$, $2x10^6$, $5x10^6$ and $1x10^6 umg^{-1}$).

Cytokines were injected (100µl) into a single knee joint of female Sprague-Dawley rats (80-100g) under brief enflurane anaesthesia and the level of hyperalgesia assessed 1, 4 and 6h later by measuring the load tolerated by the treated limb as described previously (Perkins et al., 1992). IL-1ß (1-100units u) caused a reduction in tolerated load which lasted for 30h at 100u. IL-2 (1-100u) and IL-8 (0.01-10u) also caused a reduction in tolerated load, after IL-2 (100u) animals had recovered to control levels by 6h, however after IL-8 10u animals were still hyperalgesic at 6h but had recovered by 24h. IL-6 (1-100u) had no effect on load tolerated.

One hour after intra-articular injection of IL-1ß (1u), IL-2 (10u) or IL-8 (0.1u) the load tolerated by the treated leg fell from 100g to 67±2, 66±2 and 63±3g respectively (n=8-24, $P<0.05$ Dunnett's test). Co-administration of desArg9Leu8BK (0.5-5nmol) or Hoe 140 (5pmol) with the cytokines prevented the hyperalgesia at this time point, with animals tolerating 83±3 (IL-1), 86±2 (IL-2) and 94±3 (IL-8) respectively after desArg9Leu8BK and 86±5 (IL-1), 92±3 (IL-2) and 92±2g (IL-8) after Hoe 140 ($P<0.05$, n=8-24). Co-administration of Hoe 140 with IL-1ß, IL-2 & IL-8 failed to prevent hyperalgesia developing 4h later with a reduction in tolerated load to 59±3, 60±3 and 67±4g (n=8-24, $P<0.05$) respectively. However following co-administration of desArg9Leu8BK (0.5-5nmol) hyperalgesia was still blocked 4h later with animals tolerating 84±2 (IL-1), 90±2 (IL-2) and 92±3g (IL-8) (n=8-16, $P<0.05$).

Intravenous administration of desArg9Leu8BK (10nmol/kg) or Hoe 140 (100pmol/kg) 3.5h after IL-1ß (1u), IL-2 (10u) or IL-8 (0.1u) caused a significant reversal of hyperalgesia 30min later. Animals treated with IL-1ß, IL-2 and IL-8 tolerated 90±2, 93±2, and 94±2g after desArg9Leu8BK and 90±2, 88±3 and 94±2g after Hoe 140 (n=8-24, $p<0.05$) compared to 63±2, 60±3 and 64±1g respectively after saline.

These data suggest that after intra-articular administration of IL-1ß, IL-2 and IL-8 both B1 and B2 receptors are involved in the maintenance of hyperalgesia but B1 receptors appear to play a critical role in the development of this type of cytokine-induced hyperalgesia.

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606P DIVERGENT EFFECTS OF OLVANIL IN TWO ACUTE MODELS OF NOCICEPTION IN THE MOUSE

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In high doses (>100 mg kg^{-1} sc), olvanil, a non-pungent derivative of capsaicin, has an analgesic action in thermal (Sietsema et al 1988) and chemical (Brand et al, 1990) models of nociception. In this study we report the effects of low doses of olvanil in two *in vivo* models of acute nociception.

Male TO mice (20-30g) were randomly distributed into groups of 10 and dosed s.c. with either vehicle (1% ethanol, 1% tween 80) or olvanil (1, 3 & 10 mg kg^{-1}) 180 min prior to testing in the hot-plate (50°C) or in the phenyl-p-quinone (2.5 mg kg^{-1} i.p.) induced mouse abdominal constriction (MAC) models. In some mice, capsazepine (30 mg kg^{-1}) was given 2h after olvanil. Nociceptive thresholds were measured in both tests by recording the latency to first reaction to the nociceptive stimulus. % analgesia in the MAC test was determined using the following formula: $\frac{T-C}{600-C} \times 100$ where T = test time(s), C = mean control time(s), 600 = cut off time(s). All tests were performed with the operator blind to treatment. Data were analysed for statistical differences using Students 't' test. Values are mean ± s.e. mean.

In the hot-plate model, olvanil produced a dose dependent lowering of nociceptive threshold which was found to be significantly different ($P<0.05$) from the control level (26.1 ± 0.75 s) at 3 (14.05 ± 1.3 s) & 10 mg kg^{-1} (6.45 ± 0.35 s). The dose of olvanil required to give a 50% decrease of control threshold was 3.7 (7, 1.95 C.I.) mg kg^{-1}. These data show that olvanil produces hyperalgesia to noxious heat. Conversely, in the MAC model, olvanil produced a dose dependent analgesic action, displaying a small effect at 1.0 (14.8 ± 5.7%) and larger, significant effects at 3.0 (74.4 ± 9.9%) & 10.0 (95.7 ± 4.3%) mg kg^{-1}. The latency to first constriction was found to be significantly ($P<0.05$) higher than control level (262 ± 9.9 s) at 1 and 3 mg kg^{-1} (312.4 ± 19.3 & 513.5 ± 33.4 s respectively). At the highest dose the latency in several mice reached the maximum (600 s). The dose needed to give 50% analgesia in this model was 1.9 (3.23, 1.11 C.I.) mg kg^{-1} olvanil. In both tests, capsazepine did not affect the actions of olvanil.

Although a lowering of reaction latencies has been noted for capsaicin in the rat tail immersion (50°C) test (Hayes & Tyers 1980), a hyperalgesic effect of olvanil has not been reported previously. Interestingly this hyperalgesic effect coincides with profound hypothermia (unpublished data). Further experiments are in progress to examine this phenomenon. In our MAC test we found olvanil (s.c.) to be about 10-fold more potent as an analgesic than previously reported (Brand et al, 1990) This difference may be due to our measuring the latency to first constriction, rather than the total number of constrictions.

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607P THE ACTIONS OF TACHYKININ AGONISTS ON SINGLE MOTONEURONES IN THE NEONATAL RAT SPINAL CORD PREPARATION

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The neurokinin receptor(s) which mediate the depolarization of rat motoneurones is not clear. We have studied the actions of selective tachykinin agonists on lumbar motoneurones in neonatal rat spinal cord slices using intracellular recording with the aims of identifying the receptors and ionic mechanisms involved. Intracellular recordings from putative motoneurones were obtained as described by Wang & Dun (1990). Results were obtained from 79 motoneurones with a R.M.P. of -69.5±5.9 mV, input membrane resistance of 33.1±19.3 MΩ and time constant for membrane capacitance of 6.5±3.3 ms (means±S.D.). Substance P (SP) and the selective NK_1 agonists [Sar^9, $Met(O_2)^{11}$]-SP (Sar Met SP) and substance P methylester (SPOMe), the selective NK_2 agonist GR 64349 and the selective NK_3 agonists senktide and [$MePhe^7$]-neurokinin B (MePhe NKB) were all superfused at known concentrations for 1-2 min. Their mean depolarizations (mV) are summarized in Table 1.

Table 1.	0.1 μM	0.3 μM	1 μM	3 μM	10 μM
Substance P	1.4±0.4 (5)	5.0±2.1 (3)	8.2±1.7 (19)	8.4±1.4 (24)	6.7±0.9 (18)
Sar Met SP	3.0±1.0 (2)	6.2±1.9 (5)	12.2±2.8 (10)	9.4±2.4 (8)	——
SPOMe	——	2.3±0.9 (3)	6.5±1.7 (7)	7.9±1.7 (4	——
GR 64349	——	——	2.0±1.0 (2)	2.4±0.9 (7	——
MePhe NKB	——	——	14.0±5.7 (4)	3.0 (1)	——
Senktide	——	——	2.3±0.9 (3)	7.5±5.2 (3)	±s.e.mean (n)

The depolarizations to all agonists were associated with an increase in background synaptic activity and were reduced, but not abolished, by low Ca solution or tetrodotoxin. All the agonists had effects on input membrane resistance. For SP an increase was seen in 3/6, no change 1/6 and a reduction in 2/6 experiments. SP-evoked direct depolarization was markedly reduced in 2 experiments by a low Na solution.

We have found that tachykinins have complex actions on motoneurones in the neonatal rat spinal cord slice preparation. All the selective agonists have direct and indirect actions and the direct depolarization is possibly mediated via more than one ionic mechanism.

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608P NEUROKININS RELEASED TONICALLY IN THE SPINAL CORD OF ANAESTHETIZED RATS MODULATE EXCITATORY AMINO ACID EFFICACY

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Neurokinins can modulate excitatory amino acid (EAA) effectiveness (Dougherty *et al*, 1993). We have recently reported that GR73632, a selective agonist at neurokinin$_1$ (NK_1) receptors (Hagan *et al*, 1991) enhanced responses to NMDA, AMPA and kainate (KA) relatively non-selectively, whereas GR64349, a selective NK_2 agonist, had little effect on responses to NMDA but decreased particularly those to KA (Cumberbatch *et al*, 1994). We have now examined the effects of selective NK_1 and NK_2 antagonists.

Experiments were performed on α-chloralose anaesthetized spinalized rats. Seven-barrel micropipettes were used to record from spinal neurones and to administer the following drugs by microiontophoresis: the EAA agonists NMDA (100mM in 100mM NaCl), AMPA (10/200mM) and KA (5/200mM); the NK_1 agonist GR73632 (2/50mM) and antagonists GR82334 (2/50mM) and CP-99,994 (20/20mM); and the NK_2 agonist GR64349 (2/50mM) and antagonist GR103537 (1/50mM) (Hagan *et al*, 1991; 1993); all EAA solutions were at pH 7-8 and all NK solutions at pH 4.5-5.5. EAA were ejected in regular cycles and NKs were tested for effects on the evoked responses. Drug effects are expressed as a % of the last 3 pre-drug control responses, ± SEM.

The NK_1 antagonists, as expected, reduced the enhancement of EAA responses caused by GR73632, but in addition altered EAA responses themselves. GR82334 reduced KA (to 32% control, ± 5) and AMPA (to 44% ± 6) more than NMDA (73% ± 4); n = 11-16. CP99,994 had similar effects (38% ± 6, 64% ± 6 and 80% ±6 respectively, n = 6-8). These actions were reminiscent of those of the NK_2 agonist GR64349, and suggested that block of NK_1 receptors might be revealing actions of endogenous kinins acting on NK_2 receptors. Consistent with this, preliminary tests (n = 11) indicate that on some cells the NK_2 antagonist GR103537 has effects similar to those of the NK_1 agonist, enhancing EAA responses, although the effects were more variable between cells. Combined administration of NK_1 and NK_2 antagonists has yet to be performed.

These results provide further evidence that neurokinins can modulate EAA function, and that EAA-NK interactions are receptor specific. The combined activation of NK_1 and NK_2 receptors appears to bias EAA excitation in favour of NMDA receptor mediated transmission. Under the conditions of acute electrophysiological experiments such modulation evidently occurs as a result of the basal release of endogenous neurokinins (Duggan *et al*, 1990).

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Acknowledgements: We thank the Wellcome Trust and the MRC for support.

609P THE NK_1 RECEPTOR ANTAGONIST CP-99,994 BLOCKS DURAL EXTRAVASATION AND C-fos EXPRESSION PRODUCED BY ELECTRICAL STIMULATION OF THE TRIGEMINAL GANGLION IN RATS

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The trigeminovascular system is activated during a migraine attack. Trigeminal sensory fibres project to peripheral tissues such as the dura mater that are thought to be involved in producing migraine headache pain and centrally to neurones within the trigeminal nucleus caudalis (TNC) that are involved in central nociceptive pathways. Electrical stimulation of the trigeminal ganglion (TG) evokes a neurogenic plasma extravasation in the dura that is blocked by serotonergic agonists that are clinically effective anti-migraine agents (Moskowitz, 1992) and by NK_1 receptor antagonists (Shepheard et al., 1993) and also activates neurones in the TNC as indicated by increased c-fos expression (Uhl et al., 1991). This study examines the effects of a brain-penetrant NK_1 receptor antagonist CP-99,994 and a poorly brain-penetrant $5HT_{1D}$ agonist sumatriptan on these peripheral and central markers of trigeminal activation.

Male SD rats (200 g) anaesthetised with pentobarbitone sodium (60 mg kg^{-1}, i.p.) were placed in a stereotaxic frame and bipolar stimulating electrodes placed bilaterally into both TG's. In extravasation studies, 10 min after i.v. CP-99,994 (1-1000 μg kg^{-1}), its (-)enantiomer CP-100,263 (1000 μg kg^{-1}) or H_2O vehicle (n=8-10 per group) and 5 min after a radioactive plasma marker (^{125}I BSA, 70 μCi kg^{-1}, i.v.) the right TG only was stimulated (0.6 mA, 5 ms, 5 Hz for 5 min). Plasma extravasation was measured in dural samples from both sides by counting the radioactivity in each sample. In c-fos studies, 10 min after i.v. sumatriptan, CP-99,994, CP-100,263 (1 mg kg^{-1}) or H_2O vehicle (n=5 per group) the right TG only was stimulated as before. In some animals (n=5 per group) an intra-carotid infusion of 25% mannitol was given to disrupt the blood brain barrier (BBB) 2 min. prior to sumatriptan or vehicle injection. C-fos expression in the brainstem was measured using *in situ* hybridization techniques to label c-fos mRNA (Sirinathsinghji & Dunnett, 1993). C-fos expression was located specifically within the TNC on the stimulated side. Ratios of dural plasma extravasation and c-fos mRNA expression on the stimulated/unstimulated sides were calculated. Statistical comparisons were made using ANOVA and t-tests, results were considered significant at $p < 0.05$ and are given as mean±s.e.mean

CP-99,994 produced a significant dose-related reduction in the dural extravasation ratio with an ID_{50} (dose giving 50% inhibition) of 35 μg kg^{-1}. CP-99,994 also produced a significant reduction in c-fos ratio of 39±6% compared to vehicle. CP-100,263 had no significant effect on either the extravasation ratio or c-fos ratio. Sumatriptan alone had no effect on c-fos but after mannitol pretreatment produced a significant reduction in c-fos ratio of 53±7% compared to vehicle. This study indicates that brain penetrant NK_1 antagonists such as CP-99,994 have potential anti-migraine effects through actions at central as well as peripheral sites. In contrast the $5HT_{1D}$ agonist sumatriptan blocked c-fos expression in the TNC only after disruption of the BBB, suggesting that its anti-migraine effects are predominantly peripherally mediated.

We thank our colleagues in Medicinal Chemistry M.R.L. for the synthesis of CP-99,994, CP-100,263 and sumatriptan.

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610P PAIRED-PULSE POTENTIATION DURING ETHANOL WITHDRAWAL: CHANGES IN GABA$_B$ TRANSMISSION?

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Withdrawal from chronic ethanol intake produces characteristic behavioural disturbances, and we have evidence that changes may occur in GABA$_B$-mediated transmission (Mead and Little, 1993). GABA$_B$ receptors are thought to be involved in the responses to paired stimuli in CA1 of the hippocampus (Davies *et al.*, 1990) and 2-OH saclofen has been shown to block the pre- and postsynaptic effects of baclofen (Harrison *et al.*, 1990). We have therefore investigated the effect of ethanol withdrawal on GABA$_B$ synaptic modulation using paired-pulse stimulation.

Male C57 mice (28-35g) received 24% ethanol v/v (10-14 g/kg/day) as sole fluid for 18 weeks; controls received tap water (n values 5 to 7 per treatment group). Extracellular recordings were made from transverse hippocampal slices, area CA1. The Schaffer collateral commissural pathway was stimulated using dual pulses, with a range of time intervals between 10 and 180 ms. Paired-pulse potentiation (PPP) of population spike height was determined at 1.25 x threshold for elicitation of a population spike. All measurements were made at 2, 4 and 6 h following withdrawal from ethanol (i.e. from time of dissection). The effect of the GABA$_B$ receptor antagonist, 2-OH saclofen (50 and 200 μM) was tested on PPP of population spike height at 4 h into the withdrawal period. Statistical analysis was by two-way analysis of variance. Values in the table are means ± s.e.m. of PPP calculated as second population spike height/first population spike height (expressed as percentages).

Slices from ethanol-treated mice showed significant decreases in PPP at 4h and 6h (but not at 2h) into the withdrawal period, compared with controls ($P < 0.05$ compared over pulse intervals 14 to 180 ms and 10 to 20 ms respectively). 2-OH saclofen showed no significant effect on control slices. In slices from ethanol treated animals, 200 μM 2-OH saclofen increased PPP when 10-20 ms intervals were used ($P < 0.02$), but did not alter the PPP seen with longer pulse intervals.

Table 1: Paired-pulse potentiation at representative pulse intervals, 4 h withdrawal

	Interpulse intervals (ms)									
	12	14	16	18	20	40	60	80	140	180
Controls	87±18	115±16	129±12	130±13	137±13	166±10	168±9	177±14	158±10	146±10
Controls + 200 μM 2-OH saclofen	126±15	129±14	142±17	144±13	155±12	179±18	196±16	200±25		
Ethanol withdrawal (Eth/wd)	92±12	88±11	99±6	104±3	111±3	132±9	133±8	136±11	127±9	125±12
Eth/wd + 200 μM 2-OH saclofen	116±6	122±5	122±6	128±6	129±8	136±5	137±8	138±8		

Such results may imply changes in GABA$_B$ transmission during the ethanol withdrawal period.

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611P INCREASED EXTRACELLULAR GABA AND TAURINE IN THE VENTROLATERAL THALAMUS OF RATS WITH NON-CONVULSIVE EPILEPSY

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A selectively-bred strain of rats, designated Genetic Absence Epilepsy Rats from Strasbourg (GAERS), have been validated as an experimental model of human absence epilepsy (Marescaux et al., 1992). Pharmacological evidence indicates a critical role for the $GABA_B$ receptor in the production of the spike-and-wave discharges (SWD), superimposed on a normal background EEG, that characterise this disorder (Liu et al., 1992). These SWD originate in the thalamus but $GABA_B$ receptor density and affinity in this region in GAERS is not different from the control strain (Knight and Bowery, 1992). It is possible, therefore, that raised extracellular GABA levels may be responsible, thus we have used in vivo microdialysis in the ventrolateral thalamus to examine levels of GABA and other amino-acids.

Male Wistar rats (260-360g, GAERS and non-epileptic controls) were implanted, under chloral hydrate anaesthesia (400 mg/kg^{-1}), with concentric dialysis probes in the ventrolateral thalamus (2.1P, 2.4L, 6.6V mm from bregma). On the following day, probes were perfused with artificial CSF at 0.5 µl/min. After collection of 4 x 30 min basal samples, rats from both strains received (-)baclofen (2 mg/kg^{-1}, i.p.) and six further 30 min samples were collected. In a separate series, after collection of basal samples, the GABA uptake inhibitor, (R)-N-[4,4-bis(3-methyl-2-thienyl)but-3-en-1-yl]nipecotic acid (NO 328) was added to the perfusion medium (final concentration 100 µM) and a further 4 x 30 min samples collected. The K^+ concentration of the perfusion fluid was then raised to 100 mM for 30 min, followed by perfusion with normal solution for a further 90 min. Amino-acid content of all samples was determined by HPLC.

There was no difference in basal levels of aspartate (ASP), glutamate (GLU) and glutamine (GLN) between the 2 groups:- (ASP, 0.30 ± 0.15; 0.30 ± 0.09: GLU, 1.25 ± 0.48; 1.13 ± 0.23: GLN, 22.7 ± 8.3; 40.1 ± 10.1: µM, mean ± s.e.mean, control and GAERs respectively, n=6 each group). However, basal levels of taurine (TAU) and GABA were significantly higher in GAERS:- (TAU, 1.59 ± 0.21; 2.46 ± 0.25: GABA, 0.06 ± 0.02; 0.14 ± 0.02: µM, controls and GAERS respectively, $p<0.05$, Students t-test). Administration of the $GABA_B$ agonist, (-)baclofen (2 mg/kg^{-1}), produced no changes in extracellular ASP, GLU, GLN, TAU or GABA in either GAERS or control rats (1-way ANOVA, n=6). Perfusion of NO 328 (100µM) produced no significant change in extracellular ASP, GLU, GLN, TAU or GABA, in either GAERS or control rats (1-way ANOVA, n=6). In contrast to basal levels, there was no difference in K^+ -evoked release of TAU or GABA between controls and GAERS (TAU, 448 ± 31, 551 ± 57; GABA, 243 ± 79, 235 ± 47, % of basal, controls and GAERS respectively, n=6). These data support the hypothesis that absence epilepsy may arise from excessive GABA-mediated inhibition within the thalamus caused by increased basal extracellular GABA levels. The role of taurine is as yet unclear, although it is relevant to note its structural similarity to GABA, and its interaction with both $GABA_A$ and $GABA_B$ receptors in the brain (Kontro et al., 1990).

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612P ENHANCEMENT BY MUSCIMOL OF ADENOSINE EFFECTS ON HIPPOCAMPAL POPULATION SPIKES

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There is increasing evidence that in addition to affecting K^+ and Ca^{2+} conductances, adenosine can also modify Cl^- permeability (Mager et al ., 1990). We now report a study of interactions between adenosine and muscimol in the hippocampus.

Hippocampal slices 450 μm thick were prepared from male Wistar rats (170-210 g) and superfused at 30°C with ACSF (in mM : KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, Glucose 10, $MgCl_2$ 1.2 saturated with 95% O_2/ 5% CO_2). Orthodromic extracellular recordings were made in the CA1 pyramidal cell layer following stimulation of Schaffer collateral fibers in the stratum radiatum.

Adenosine and muscimol induced a concentration dependent reduction in the amplitude of orthodromic potentials. Maximum effects were seen at 20 and 10 μM respectively. In order to isolate effects on chloride channels and exclude the involvement of potassium channels,experiments were performed in the presence of 1mM barium. Barium alone induced an enhancement in population spike amplitude by 27.36% ± 0.87 (mean± s.e.m., P≤0.001, n=5 Student's t-test). In the presence of 8-Cyclopentyl-1,3-dimethylxanthine (CPT, A1 antagonist) 100 nM this effect was decreased to 9.56%±0.46 (P ≤0.001,n=5) suggesting that barium was acting by blocking adenosine-activated K channels. Barium has previously been shown to block adenosine-activated K conductance postsynaptically (Thompson et al., 1992). In the presence of barium muscimol,1 μM, reduced spike amplitude by 14.94% ± 0.60 (P≤0.001, n=5) while adenosine 2 μM was ineffective. The combination of muscimol 1 μM with adenosine 2 μM reduced spike size by 31.30% ± 1.22 (P≤0.001, n=5). Interestingly, this reduction was decreased in CPT 100 nM to 19.34% ±.050 (P≤0.001,n=5).

The results suggest an interaction between muscimol and adenosine which may involve A1 receptors and which may occur at the level of chloride channels.

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613P ON THE MECHANISM OF THEOPHYLLINE SUPPRESSION OF ANTIDROMIC POPULATION SPIKES IN RAT HIPPOCAMPUS

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We have previously noted that theophylline depressed the amplitude of antidromically elicited secondary spikes in the rat hippocampus bathed in calcium-free medium (Hosseinzadeh & Stone, 1994). In the present work we have attempted to clarify the mechanism of this phenomenon.

Hippocampal slices 450 μm thick were prepared from male Wistar rats (170-210 g) and superfused at 30°C with ACSF (mM: KH_2PO_4 2.2, KCl 2, $NaHCO_3$ 25, NaCl 115, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 10) saturated with 5% CO_2 in oxygen. Recordings of orthodromically evoked synaptic activity were made in the CA1 pyramidal cell layer following stimulation in stratum radiatum. Antidromic responses were obtained by stimulation of the alveus in medium containing nominally zero or 0.1 mM calcium.

Adenosine at 50 μM abolished the orthodromic population potential. Theophylline, 50 μM and 8-cyclopentyl-1,3-dimethylxanthine, (CPT) 100 nM decreased the effect of adenosine to 69.93% ± 3.25 (mean ± s.e.m., Student's t-test) n=4, P<0.001, and 22.77% ± 3.22 (n=6, P<0.001) respectively. Theophylline and CPT alone increased spike size by 21.17% ± 6.24 (n=4, P<0.05) and 5.19% ± 3.57 (n=6, n.s.) respectively.

In calcium-free medium theophylline and CPT decreased secondary antidromic spike size by 50.61% ± 6.79 (n=14, P<0.001) and 50.56% ± 11.45 (n=4, P<0.05) respectively. Adenosine at 200 μM and baclofen 5 μM, which alone had no effect on secondary spike size, both prevented the inhibitory action of the xanthines. Adding 0.1 mM calcium similarly had no effect alone but reduced theophylline depression to 16.3% ± 1.49 (n=5, P<0.001). Carbamazepine, 50 μM decreased secondary spike size by 17.65% ± 4.87 (n=6, P<0.05) and significantly reduced the inhibitory activity of theophylline (n=6, P<0.05).

The results indicate that several agents can prevent xanthine depression of antidromic population spikes. A common feature of these agents is their ability to modulate calcium fluxes and/or stabilise neuronal membranes, and it is possible that the demonstrated activity of xanthines reflects an interaction with one or both of these phenomena.

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614P EFFECT OF ADENOSINE A_1 RECEPTOR AGONISTS ON THE SEIZURES INDUCED BY THE POTASSIUM CHANNEL BLOCKER 4-AMINOPYRIDINE IN MICE

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Adenosine agonists have complex effects on seizure threshold. They are anticonvulsant in most models of epilepsy, however, proconvulsant effects have also been reported (Klitgaard et al., 1993). This study investigates the effects of the adenosine A_1 receptor agonists CPA (N-cyclopentyladenosine) and R-PIA (R-N-(phenylisopropyl)adenosine) on the clonic and tonic seizures induced by the potassium channel blocker 4-aminopyridine (4-AP) in mice. The less active (+)-enantiomer of R-PIA (S-PIA) has been investigated for comparison. Male NMRI mice (20-25g; n=6-8) were injected with vehicle (5% chremophore) or different doses of the adenosine agonists 30 (i.p.) or 15 (i.c.v.) min before treatment with 4-AP (12 mg/kg s.c.). Mice were observed in the following 15 min period for the appearance of clonic and tonic convulsions. Results are expressed as ED_{50} values (i.e. the dose of drug preventing seizures in 50% of the animals). The numbers in brackets represent 95% confidence limits.

Table 1 Anticonvulsant effects of adenosine A_1 receptor agonists on the clonic seizures induced by 4-AP in mice

Treatment	ED_{50} (mg/kg i.p.)	ED_{50} (µg/mouse i.c.v.)
CPA	1.7 (0.9 - 3.1)	4.0 (1.7 - 8.0)
R-PIA	2.1 (1.0 - 4.9)	9.7 (1.7 - 26.6)
S-PIA	13.9 (5.4 - 47.5)	Not tested

The ED_{50} values against tonic seizures were the same as those shown above with the exception of R-PIA i.p. (ED_{50}: 1.5 (0.6 - 5.7).

Thus the results of this study show that the adenosine A_1 agonists CPA and R-PIA (given either i.p. or i.c.v.) inhibit the clonic and tonic convulsions induced by 4-AP in mice. Stereoselectivity was observed since S-PIA was less active than R-PIA. The doses of CPA and R-PIA blocking 4-AP-induced seizures were lower than those required to block the convulsions induced by pentylenetetrazol (clonic ED_{50} values of 5.3 and >10 mg/kg i.p. respectively; Klitgaard et al., 1993) or maximal electroshock (50 mA via corneal electrodes; tonic ED_{50} values: 4.2 and 9.2 mg/kg i.p.). The mechanisms underlying the convulsant effects of 4-AP are thought to involve release of excitatory amino acids e.g. glutamate (Yamaguchi et al., 1993). Since adenosine inhibits glutamate (and aspartate) release in vitro (Corradetti et al., 1984) and since CPA and R-PIA block glutamate-induced seizures in mice (clonic ED_{50} values: 0.4 and 1.1 mg/kg i.p.; Klitgaard et al. 1993) it may be postulated that the anticonvulsant action of adenosine A_1 agonists on 4-AP-induced seizures may be mediated, at least in part, by their modulatory effects on levels of excitatory amino acids.

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615P THE NOVEL ANTIEPILEPTIC DRUG LEVETIRACETAM (ucb L059) APPEARS TO ACT VIA A SPECIFIC BINDING SITE IN CNS MEMBRANES

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Levetiracetam ((S)-α-ethyl-2-oxo-pyrrolidine acetamide, ucb L059) is a novel anticonvulsant agent with a unique profile of action (Gower et al.,1992; Löscher and Hönack, 1993), presently in clinical development. The effect of this compound is highly stereoselective since ucb L060, the (R) stereoisomer of ucb L059, lacks anticonvulsant activity. A receptor binding profile revealed that ucb L059 did not recognize any of a broad variety of neurotransmitter receptors. In order to clarify its mechanism of action, we investigated the presence of specific binding sites for ucb L059 in the rat CNS.

Male Sprague-Dawley rats (250-300 g) were killed by decapitation. Binding assays were performed on crude membranes (300 µg protein/assay) at 25 °C in 50 mM Tris-HCl buffer (pH 7.4), containing 2 mM $MgCl_2$ and 10 nM [^{3}H]L059 (36 Ci/mmol). Specific binding was found to be reversible, saturable and stereoselective. Equilibrium binding was reached within 30 min, and complete dissociation was achieved within 5 min. Results obtained at equilibrium suggest that the tracer labels a single class of binding sites. The K_D value was between 1 and 2 µM in every brain region investigated (hippocampus, cortex, cerebellum and striatum), and the Bmax values varied from 5 to 10 pmol/mg protein. ucb L060 displayed at least 500 times less affinity for these sites. Interestingly, the binding of [^{3}H]L059 was confined to the central nervous system; no specific binding was observed in a range of peripheral tissues including heart, kidneys, spleen, pancreas, adrenals, lungs and liver.

The commonly used antiepileptic drugs carbamazepine, phenytoin, valproate and clonazepam, as well as the convulsant agents picrotoxin and bicuculline did not displace [^{3}H]L059 binding. However, pentylenetetrazole competed with [^{3}H]L059 with a pKi of 4.1, a value relevant to the convulsant dose used *in vivo*. Structurally related compounds including piracetam and aniracetam, also displaced [^{3}H]L059 binding with a rank order of potency which correlated with their anticonvulsant activity in the audiogenic mouse test.

These results support a role of this binding site in the anticonvulsant activity of ucb L059 and substantiate the singular pharmacological profile of this compound. However, this site remains to be further characterized. In this regard, it has been successfully solubilized in digitonin and its purification is in progress.

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616P EVALUATION OF THE COMPARATIVE EEG SPECTRAL PROFILES OF WIN55212-2, A STRUCTURALLY NOVEL CANNABINOID MIMETIC, AND THE SYNTHETIC CANNABINOID LEVONANTRADOL IN THE RAT

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Cortical EEG recordings have been used to study the pharmacology of tetrahydrocannabinol (THC) and related cannabinoid in several animal species (Matsuzaki *et al.*, 1987). In this study the effects of WIN55212-2, an aminoalkylindole possessing stereoselective affinity for the cannabinoid receptor (D'Ambra *et al.*, 1992), on spontaneous EEG activity and behaviour were assessed and compared to the synthetic cannabinoid levonantradol.

Male Sprague-Dawley rats (250-300g) were anaesthetized and prepared with frontoparietal cortical EEG electrodes and an external jugular vein catheter. Following 3-5 days recovery, the rats (n=5 per dose) were injected with WIN55212-2 (0.125-0.5 mg/kg, i.v.) or levonantradol (0.0625-0.25 mg/kg, i.v.). EEG activity was monitored continuously until the onset of slow-wave sleep. Off-line spectral analyses were performed on selected pre- and post-drug EEG epochs. During wakefulness, WIN55212-2 caused marked disruptions in normal EEG patterns and over behaviour which were dose- and time-dependent. The most prominent EEG change was the immediate induction of high-amplitude synchronized bursts (SBs) resulting in an increase in EEG spectral power approaching 500% above control. The EEG SBs, which persisted for 21 ± 3 min at the highest dose tested, were distinguished by dual spectral frequency peaks: an initial large-amplitude peak centred about 4.7Hz, and a secondary small-amplitude peak centred about 9.5Hz. In contrast, the spectral power measured in the awake desynchronized EEG which emerged immediately after the EEG SBs was significantly reduced, decreasing to 77% of baseline. The EEG changes were associated with a complex behavioural syndrome consisting of a mixture of sedation with flaccid posture (primarily during the EEG SBs) and arousal. In separate experiments the inactive (S) enantiomer of WIN55212-2, WIN55212-3 (0.5 mg/kg, i.v.), had no effect on the EEG or behaviour of the rat (n=5). Qualitatively, levonantradol produced a similar EEG and behavioural profile. However, in addition to being more potent and longer acting than WIN 55212-2. preliminary analyses of the levonantradol-induced EEG SBs showed them to be distinguished by only a single spectral peak centred about 5.1Hz.

WIN55212-2 produces EEG and behaviour changes in rats consistent with a cannabinoidmimetic profile and stereoselectivity for the cannabinoid receptor, yet apparently distinguishable from levonantradol on the basis of the frequency profiles of the EEG SBs.

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617P EFFECTS OF POTASSIUM CHANNEL BLOCKERS ON EXTRACELLULAR LEVELS OF SEROTONIN AND DOPAMINE IN THE STRIATUM OF CONSCIOUS RATS: A MICRODIALYSIS STUDY

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The role of Potassium (K) channels in controlling membrane potential and excitability of neurones is well established (Hille 1984). K channels are postulated to participate in the control of neurotransmitter release from nerve terminals by dictating the threshold and time course of action potentials invading the terminal. The exact role of K channels in the regulation of neurotransmitter release is, however, unclear. In the present study we examined the effects of four K channel blockers [4-aminopyridine (4AP), tetraethylammonium (TEA), mast cell degranulating peptide (MCDP) and apamin] on extracellular levels of serotonin (5-HT) and dopamine (DA) in the rat striatum using *in vivo* microdialysis.

24 hours post implantation of microdialysis guide cannulae into the striatum of anaesthetised male Sprague-Dawley rats (280-360g), dialysis probes were inserted for measurement of extracellular levels of 5-HT and DA (Routledge *et al*, 1993). Probes were perfused with artificial cerebrospinal fluid pH7 (1µl/min) and following a 3 h stabilisation period samples were collected at 20 min intervals. Three control samples were taken prior to infusion of K channel blockers into the striatum, via the dialysis probe, every 30 min in a cumulative manner. Infusion of TEA (1µM-10mM) induced a concentration-related and significant ($p<0.05$) increase in extracellular levels of DA the maximum effect being 500±193% of preinfusion control values following infusion of 1mM TEA. Concomitantly, TEA significantly ($p<0.05$) increased extracellular 5-HT levels, however, this response was smaller, reaching a maximum of 222±61% of preinfusion control values following infusion of 10mM TEA. Infusion of MCDP (100nM-100µM) induced a significant ($P<0.05$) concentration-dependent increase in DA levels producing a maximal response that was 219±84% of preinfusion control values following 10µM MCDP. MCDP (up to 100µM) had no significant effect on extracellular levels of 5-HT. Infusion of apamin (100nM-100mM) induced a significant ($p<0.05$) and concentration-dependent increase in extracellular levels of 5-HT, reaching a maximum of $1x10^5$% of preinfusion control levels following infusion of 1mM apamin. Following infusion of 100µM and 1mM apamin animals exhibited behavioural changes characteristic of the 5-HT syndrome (i.e. forepaw treading, head shakes, flat body posture) (Jacobs, 1976). Apamin had no significant effect on extracellular DA levels except at the highest concentration (1mM), at which convulsions were induced. Infusion of 4AP (1µM-1mM) induced a significant ($P<0.05$) and concentration-related increase in extracellular levels of DA but had no effect on 5-HT release. Infusion of 4AP at a concentration of 10µM increased extracellular DA to 148±28% of preinfusion of control values and this level was maintained throughout subsequent infusions.

In conclusion, the increase in DA levels induced by 4AP, TEA and MCDP suggest that DA release may be controlled by voltage-dependent K channels. These K channels may be delayed rectifier (DR) and transient current (e.g. I_A) K channels, though the low concentrations of 4AP, TEA and MCDP required suggests the involvement of dendrotoxin sensitive DR-like currents. In contrast, the selective and marked increase in extracellular levels of 5-HT induced by apamin suggests that 5-HT release may be regulated by small amplitude Ca^{2+}-activated K channels.

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618P EFFECT OF CHRONIC L-DOPA TREATMENT ON GENE EXPRESSION OF PREPROENKEPHALIN, PREPROTACHYKININ AND GLUTAMIC ACID DECARBOXYLASE IN THE STRIATUM OF 6-HYDROXYDOPAMINE LESIONED RATS

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Unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway causes an increase of striatal enkephalin (ENK) and glutamic acid decarboxylase (GAD), but a fall in substance P (SP) level. Chronic L-3,4-dihydroxyphenylalanine(L-dopa) treatment of 6-OHDA lesioned rat further elevates enkephalin (ENK) content and glutamic acid decarboxylase (GAD) activity but reverses the decrease in substance P (SP) (Enbger et al., 1991). We now report on the effect of chronic L-dopa treatment on the gene expression of preproenkephalin (PPE), preprotachykinin (PPT) and GAD mRNAs in the striatum of sham-lesioned and 6-OHDA lesioned rat.

Unilateral or sham lesions of the nigrostriatal pathway in female Wistar rats were produced by stereotaxic injection of 8μg of 6-OHDA or 0.9% saline into the left medial forebrain bundle (AP-2.2, L+1.5, V-8.0; Pellegrino et al., 1979). L-dopa (200mg/kg/24h) or carbidopa (CD) (25mg/kg/24h) was administered in drinking water to 6-OHDA-lesioned and sham-lesioned rats for 8 weeks beginning 2 weeks after the lesion. Subsequently, cryostat sections (12μm) through the medial striatum (AP 0.2mm from bregma) were incubated with ^{35}S-labelled oligodeoxyribonucleotide probes hybridizing with PPE, PPT or GAD mRNA. Quantitative evaluation of autoradiogram of the hybridization signal was undertaken by computerized densitometry.

Table 1 Effect of L-dopa treatment on gene expression of PPE, PPT and GAD mRNAs (nCi/mg)

	PPE mRNA		PPT mRNA		GAD mRNA	
	Lesion	Intact	Lesion	Intact	Lesion	Intact
6-OHDA lesion+L-dopa(n=6)	1.763±0.099#	1.310±0.084	1.185±0.100	1.274±0.089	0.800±0.0550#	0.617±0.023
6-OHDA lesion+CD(n=6)	2.210±0.069*	1.288±0.063	0.695±0.052*	1.326±0.083	0.844±0.028†	0.626±0.018
Sham lesion+L-dopa(n=6)	1.342±0.071	1.324±0.056	1.268±0.300	1.296±0.11	0.631±0.028	0.630±0.023
Sham lesion+CD(n=6)	1.366±0.078	1.305±0.075	1.323±0.088	1.300±0.11	0.636±0.023	0.632±0.009

Statistics by two-way ANOVA and post hoc comparison by Dunn's multiple test: *P<0.001, †P<0.01, #P<0.05 vs intact side and controls

Sham lesion had no effect on PPT, PPE and GAD mRNA expression (Table 1). 6-OHDA lesioning(with CD as control)markedly increased PPE mRNA and modestly increased GAD mRNA, but significantly decreased PPT mRNA level in the denervated striatum compared to the intact side and control animals. L-dopa treatment of 6-OHDA rat reduced the lesion-induced upregulation of PPE mRNA and tended to reduce the lesion-induced upregulation of GAD mRNA. L-dopa completely reversed the lesion-induced downregulation of PPT mRNA but not effect in sham-lesioned rats.

These results further demonstrate that, at the molecular levels, chronic L-dopa treatment differentially affects lesion induced changes in PPE and PPT mRNA. This may reflect an imbalance in the activity of the striatonigral and striatopallidal output pathways of relevant to the control of motor symptom in parkinson's disease.

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619P INFLUENCE OF SCAN REPETITION RATE UPON RESPONSES MEASURED BY FAST CYCLIC VOLTAMMETRY: ANALYSIS OF SAMPLING ERROR

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Fast cyclic voltammetry (FCV) has been used to measure electrically-evoked dopamine (DA) efflux in brain slices. Scan repetition rates are typically in the range 2-10 Hz. It has been found that the scan repetition rate influences the observed peak efflux and rate of decay. The present study examines the effect of scan repetition rate on the observed efflux profile. Experiments were performed in slices of rat nucleus accumbens as previously described (Millar et al., 1992).

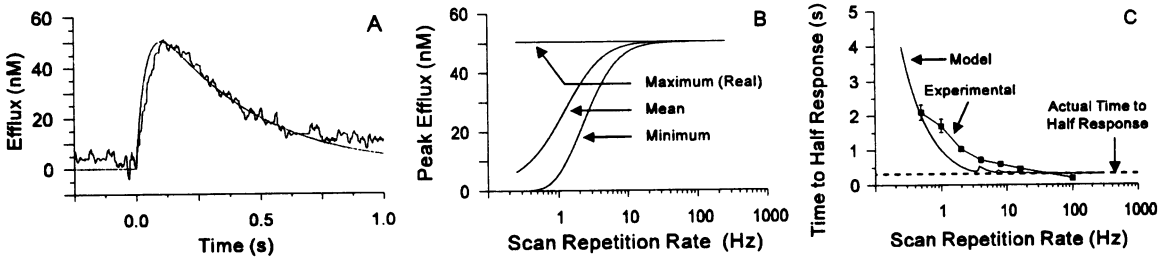


Figure 1. Continuous scan cyclic voltammetry (CSCV; Millar et al., 1992) was used to monitor DA efflux evoked by a single electrical pulse (10 mA, 0.1 ms). CSCV measures the extracellular DA level at 100 Hz. The resulting efflux profile was then used as a template for a mathematical model (Figure 1A). The model efflux profile consisted of an exponential rise phase followed by a monophasic exponential decay phase. The effect of asynchronously sampling the model profile at scan repetition rates in the range ¼-256 Hz was then calculated. The results are shown in Figures 1B & 1C. As shown, the predicted accuracy of sampling increases with repetition rate.

The validity of this mathematical simulation was tested experimentally. DA efflux evoked by single electrical pulses was measured using FCV (repetition rate ½-16 Hz) and CSCV (100 Hz). The time for the efflux to decay to half of the peak efflux (T_h) was determined and the correspondence between the predicted and actual data is shown in Figure 1C. There is a general agreement between the two curves.

This study shows that the scan repetition rate has a notable effect on the responses recorded. At low scan repetition rates, there is a tendency to under-estimate the peak efflux and to over-estimate the T_h. At high frequencies, measurement error of this type is negligible. However, at these frequencies, other factors may influence the observed profile (see Trout et al., this meeting).

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620P A COMPARISON BETWEEN TWO *IN VITRO* METHODS FOR MONITORING ENDOGENOUS DOPAMINE RELEASE

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Superfusion (SUP) or sequential incubation (SEQ) of brain tissue in various media are widely used *in vitro* methods for monitoring neurotransmitter release. We compared the methods by measuring the effects of various agents on the release of endogenous dopamine (DA) in rat striatum.

Striatal slices (400-500 μm) were either superfused with, or sequentially incubated in, oxygenated Krebs-Ringer at 34° containing the agents to be tested. In the SUP experiment, 2 slices per chamber were superfused at 0.2 ml/min and fractions collected over 2 min. In the SEQ method, 2 slices were transferred between incubation tubes containing 1 ml solution, at 5 min intervals. The effects of K^+ (25, 50 and 75 mM, dual stimulation, S1 and S2, at a 40 min interval) veratridine (Ver, 25 μM) 4-aminopyridine (4-AP, 100 μM) tetrodotoxin (TTX, 1 μM) or calcium-free (-Ca) buffer on DA release were studied. DA was quantified by HPLC with electrochemical detection. Changes in DA release were expressed as a percent of basal release. The effects of treatment and method were interpreted by regression analysis.

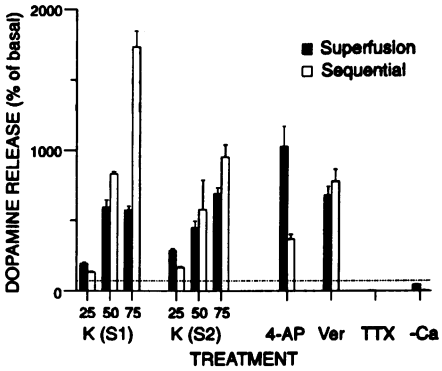


Figure 1. Effect of treatment (see text) on dopamine release (% of basal) from striatal slices. Broken line is basal release (100%). Values are mean ±s.e.mean, n=6.

Mean basal DA release (±s.e.mean, n=84) was 1.32±0.06 (SUP) and 1.82±0.10 (SEQ) pmol/ml/mg protein ($P<0.001$). Figure 1 shows the effects of treatments using the two methods. All treatments caused a significant change in DA release ($P<0.02$). K^+ caused a dose-related increase in DA release; at 25 mM, it was less for SEQ ($P<0.001$), whereas at 75 mM it was greater for SEQ ($P<0.001$). SEQ resulted in a lower response to 4-AP ($P<0.001$) but, no significant difference was seen with Ver. TTX caused a highly significant decrease in spontaneous DA release in both methods. DA release was decreased in calcium-free buffer, the effect being significantly greater in SEQ ($P<0.001$).

The data show that induced changes in spontaneous endogenous dopamine release can be quantified using either method, but SEQ has the advantage that approximately half the number of samples are required. The reasons for the quantitative differences between the methods in the response to some treatments are unknown and require further investigation.

621P EFFECTS OF SUBSTANCE P AND TWO OF ITS FRAGMENTS ON ENDOGENOUS DOPAMINE RELEASE FROM STRIATAL SLICES

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Petit and Glowinski (1986) showed substance P (SP, 0.001-0.1 μM) stimulated the spontaneous release of [3H]-dopamine (DA) from rat striatal slices. However, Guevara Guzman *et al.* (1993) have shown that, *in vivo,* the striatal infusion of SP had no effect on the release of endogenous DA. This study investigated the effects of SP and its 1-7 (SP 1-7) and 5-11 (SP 5-11) fragments on the spontaneous release of endogenous DA.

Striatal slices were superfused with oxygenated Krebs-Ringer at 34°C (Khan *et al.*, 1993). Fractions were collected over 2 min. After 4 min, peptide solutions (0.01-10 μM), were infused for 10 min, and samples collected for a further 10 min. DA was quantified by HPLC with ECD. Changes in DA were expressed as a percent of basal release.

Basal release of DA was 1.45 ± 0.04 pmol/ml/mg protein, n=144. One way ANOVA did not show any concentration dependent effects ($F(3,20) = 2.99$, 0.07 and 2.1, all ns, for SP, SP 1-7 and SP 5-11, respectively.) (Figure 1) However, at the lowest concentration, SP significantly reduced the spontaneous release of endogenous DA.

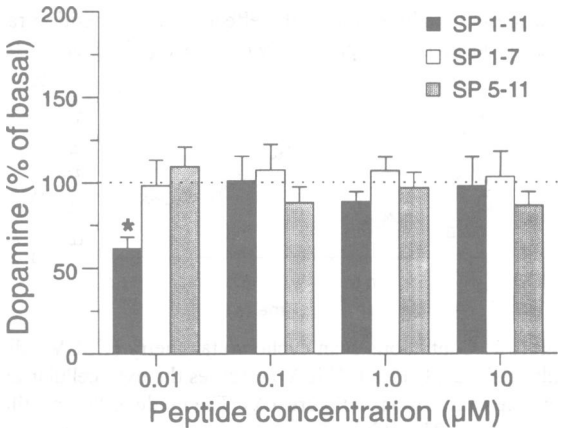


Figure 1. Effect of SP and its fragments on the release of DA from striatal slices. Values are means +s.e.mean of 6 determinations. *Significant from basal ($P<0.01$)

The SP data support the results of Guevara Guzman *et al.* (1993) but not those of Petit and Glowinski (1986). This contradiction may result from differences in experimental conditions, e.g. [3H]-DA vs. endogenous. Additionally, SP fragments, reported to modify striatal DA release after nigral administration (Reid *et al.*, 1990), produced no effects in our model.

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622P BEHAVIOURAL PROFILE OF ACTH$_{(1-39)}$: DIFFERENTIAL EFFECTS OF HALOPERIDOL AND RITANSERIN

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Adrenocorticotrophic hormone ACTH$_{(1-39)}$ is a straight-chain polypeptide hormone of 39 amino acid residues which induces excessive grooming, penile erections, whole body shakes, stretching and yawning after intracerebroventricular (ICV) injection in rats (Gispen et al., 1975). We have therefore injected ACTH$_{(1-39)}$ by the ICV route in mice and tested the effects of a dopamine-2 receptor antagonist haloperidol and a selective 5-HT$_{2A}$/5-HT$_{2C}$ receptor antagonist ritanserin, since these agents differentially blocked the related behaviours induced after ICV administration of α-melanocyte stimulating hormone (α-MSH, (N-acetylSer[1])-ACTH$_{1-13}$) in mice (Dursun & Handley, 1992).

Male MF1 mice (20-25g, N=6 / treatment) received ACTH$_{(1-39)}$ in saline ICV (Brittain & Handley, 1967) and were analysed from videotapes (blind to treatment) for the ensuing 60 min. Grooming of the body, head and face, and fore-paw licking were recorded as time; head-shakes, yawning and stretching were recorded as number. All the behaviours were dose-dependent within the dose-range tested (0.1-20μg) except head-shakes which showed a bell shaped dose-response after the doses 10 to 20μg.

Ritanserin (1.0 mg/kg) and haloperidol (0.5 mg/kg) ip 30 min before 0.5μg ACTH$_{(1-39)}$ ICV caused the following changes:-

TABLE 1 Differential effects of ritanserin and haloperidol on ACTH$_{(1-39)}$ induced behaviours

	Grooming of the Head & Face	Rest of the Body	Fore-paw Licking	Yawning	Stretching	Head-shakes
	(Control vs drug-treated values: Means ± s.e. mean)					
	Minutes				Numbers	
Ritanserin	3.6±1 vs 3±1.1	8±1.8 vs 7.6±2.3	3.2±1.3 vs 2.9±1.2	17±2.6 vs 7.4±2.1*	23.2±4.7 vs 10.2±3.5*	18.2±3.4 vs 7±2.4*
Haloperidol	3.3±1.3 vs 2.2±0.6	7.6±1.7 vs 3.6±0.7*	2.7±1.2 vs 1.9±0.8	17.6±3.2 vs 7.5±2.6*	24.4±3.2 vs 9.8±2.4*	21.6±4 vs 10.2±3.5*

(* = P<0.05, Student's t test)

Thus each agent had different effects on individual movements and the pattern of antagonism by ritanserin was different from that produced by haloperidol. This suggests that 5-HT and dopamine may be differentially involved in modulating these movements when induced by ACTH$_{(1-39)}$.

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623P HETEROGENEOUS EFFECTS OF (+)-AMPHETAMINE ON DOPAMINE OVERFLOW IN THE RAT CAUDATE PUTAMEN

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Dopamine (DA) overflow in response to electrical stimulation of rat striatal brain slices (CPu) displays regional heterogeneity (Patel et al., 1992). Recently we have shown that it is possible to distinguish two populations of DA overflow sites in rat CPu with differing sensitivity to the D_2 autoreceptor agonist, quinpirole (Iravani & Kruk, 1992). Using fast cyclic voltammetry in CPu slices, we have now examined the effect of (+)-amphetamine (Amp) on electrically stimulated DA overflow and stimulation-independent DA "displacement" from these two populations of DA overflow sites. The methods used have been described (Patel et al 1992), except that Amp (10mM, 1.0s; 10 psi.) was pressure ejected from a 5μm diameter pipette 20 μm from the working electrode. When DA overflow following single pulse (*1p*) electrical stimulation (0.1ms; 20v) was compared with DA overflow following a burst of 20 pulses applied at 50Hz (*20p*/50Hz), two populations of sites could be identified. "Low ratio" sites had DA efflux ratios of {*20p*/50Hz:*1p*} of 1.4 ± 0.06 (mean ± s.e.m; n=9;). "High ratio" sites had ratios of 3.4 ± 0.2 (n=9;). Amp immediately increased the level of DA from all recording sites, producing maximal extracellular concentrations of 890 ± 69 nM, (mean ±s.e.m, n=7), at high ratio sites and 856 ± 154 nM, (n=9) at low ratio sites. Presence of 1μM sulpiride did not affect DA changes in either site. At all sites, 4 min after pressure ejection of Amp there was a marked reduction of DA overflow following single-pulse stimulation. At low ratio sites; control: 61 ± 8.21 nM; post Amp: 24 ± 11.77nM; (n=8 p=0.02). At high ratio sites; control: 82.9 ± 16.8nM; post Amp: 45 ± 10.85 nM; (n=6; p=0.09). Five min later (9 min after Amp) each site was stimulated with *20p*/50Hz. In low ratio sites, DA overflow was increased (control: 122.3 ± 20nM; Amp: 250 ± 40nM; paired t-test p=0.01 n=8). In high ratio sites however, DA overflow was not significantly reduced (control:320 ± 86nM; Amp: 266 ± 70nM; paired t-test, n=6, n.s.)(see Fig. 1). Presence of 1 μM sulpiride prevented the Amp-evoked reduction of *1p* -evoked DA overflow in both sites. Amp-induced potentiation of DA overflow by *20p*/50Hz in low ratio sites was unaffected by sulpiride. Locally applied Amp caused equivalent displacement of DA at all sites, and reduced *1p* stimulated DA overflow in both high and low ratio sites in a sulpiride sensitive fashion. This indicates that the displaced DA is acting on D_2 autoreceptors. The sulpiride-insensitive potentiation of DA overflow evoked by *20p*/50 Hz in low ratio sites may indicate a differential effect of Amp on DA uptake at these sites. Such an interpretation is in accord with the observations of Wieczorek & Kruk (1994), who examined the actions of Amp in the CPu and nucleus accumbens.

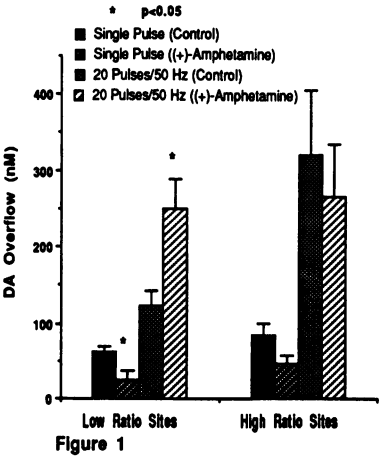


Figure 1

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624P DIURNAL VARIATION IN NMDA-STIMULATED STRIATAL DOPAMINE EFFLUX: AN *IN VIVO* MICRODIALYSIS STUDY

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Dopamine (DA) release in the rat striatum shows circadian variation which correlate to motor activity (O'Neill & Fillenz, 1985). Cortical glutamatergic input into the striatum is involved in the control of striatal DA release (Chéramy *et al*, 1991). *In vivo* microdialysis studies suggest a stimulation of striatal DA efflux by the selective glutamatergic agonist, N-methyl-D-aspartic acid (NMDA). Whether this response is influenced by circadian variation is not known. We now report that time of day produces a variation in NMDA-stimulated striatal DA efflux.

Male Wistar rats (180-220g, habituated to a standard 12 hour light/dark cycle) were used. Microdialysis probes (Whitehead *et al*, 1992) were implanted into the striatum (A +7.0 L +3.0, relative to lamda, V -1.5 from the zero plane) under halothane anaesthesia. Microdialysis was performed at least 24 hours after surgery, at either 11.00h (AM group) or 16.00h (PM group) under halothane anaesthesia. A magnesium-free artificial ECF was perfused at a rate of 1.5 µl/min for 1 hour before 15 min samples of dialysate were collected into 10µl 0.5M acetic acid. After 135 min, perfusion was switched to artificial ECF containing 1 mM NMDA for 30 minutes, before reperfusion with normal ECF for a further 45 min. Samples were analysed for DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) content by HPLC-ECD. Data is presented as mean percentages of the basal efflux (mean concentration in the first 4 samples).

Basal efflux of DA was not different between the AM and PM groups (109±10 and 131±17 fmol/15 min respectively, mean±s.e.mean; Dunn's Test, n=5 for each group). Perfusion with 1 mM NMDA evoked a marked increase in DA efflux in the AM group (maximal at 4863±1070% of basal efflux), but not in the PM group, where NMDA produced a trend towards decreased DA efflux (minimum at 70±7.6% of basal), ($P<0.05$ two-way ANOVA; Figure 1). Decreased efflux in DOPAC (minimum 19±2.9% AM, 35±3.4% PM) and HVA (minimum 17±3.5% AM, 50±6.4% PM) was seen in both groups in response to NMDA ($P<0.05$ one-way ANOVA), although there was no difference in response between groups.

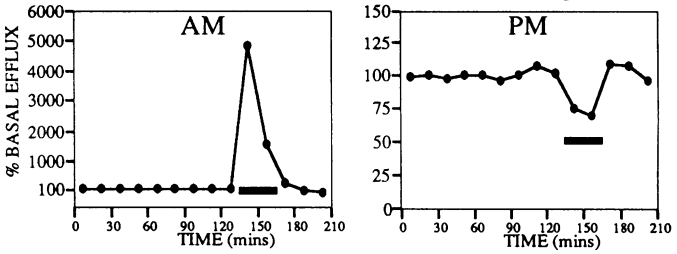


Figure 1. Effect of perfusion artificial ECF containing 1mM NMDA (bar) on striatal DA efflux in AM and PM groups. Data are mean percentages of basal efflux, n=5 for each group.

In conclusion, the present study found that basal striatal DA efflux was independent of the time of day studied. In contrast, striatal DA efflux stimulated through the NMDA receptor was subject to marked diurnal variation.

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625P THE EFFECTS OF ANTIPSYCHOTIC DRUG ADMINISTRATION ON GLUTAMATE-STIMULATED [^{3}H]DOPAMINE RELEASE FROM RAT FRONTAL CORTEX

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Jones *et al.* (1993) reported that glutamate stimulates the release of [^{3}H]dopamine from slices of rat frontal cortex. Since abnormalities of frontal cortical glutamate are implicated in schizophrenia (Reynolds 1992), we have examined the effects of two antipsychotic drugs on this system after a single dose and after 21 days treatment. Male Wistar rats (starting weight 200g) were given i.p. injections of either clozapine (25mg/kg), haloperidol (1.5mg/kg) or 0.9% saline as control. Animals were killed 20 h after the last dose and frontal cortical glutamate stimulated dopamine release determined (Jones *et al.*, 1993). This involved the tissue being sliced at 350 µm intervals in two perpendicular directions and placed in superfusion chambers after incubation with [^{3}H]dopamine. The tissue was then superfused with Krebs-phosphate buffer (400 µl/min) for 30 min before basal samples were collected and the tissue stimulated with two 2 min pulses of 1mM glutamate 18 min apart (S1, S2). The dopamine uptake inhibitor, mazindol (10µM), was present during the collection period.

Table 1. The effects of antipsychotics on glutamate stimulated [^{3}H]dopamine release

	ACUTE			CHRONIC		
	Control (n=23)	Clozapine (n=17)	Haloperidol (n=19)	Control (n=33)	Clozapine (n=32)	Haloperidol (n=31)
MEAN BASAL	1.40 (0.03)	1.24 (0.03)**	1.16 (0.04)**	1.35 (0.03)	1.24 (0.02)*	1.15 (0.03)**
S1	2.82 (0.32)	2.17 (0.29)	2.45 (0.23)	3.36 (0.28)	2.99 (0.24)	3.66 (0.28)
S2	1.87 (0.19)	1.14 (0.14)*	0.89 (0.16)**	1.89 (0.25)	1.73 (0.15)	1.84 (0.17)

Values are expressed as mean (s.e. mean) percentage of total tissue radioactivity at time of fraction collection.

* $p<0.05$; ** $p<0.001$ for Students t test vs. control

Neither drug, administered either acutely or chronically, affected the response to the first stimulation by glutamate (peak S1 measured as release above basal over a ten min period after stimulation). Acutely both drugs significantly reduced the response to the second pulse of glutamate (S2). In each case the drugs significantly reduced mean basal release (calculated from points prior to each stimulation), haloperidol having a significantly greater effect than clozapine ($p<0.05$). As shown previously (Jones *et al.*, 1993), the second response to glutamate was always significantly smaller than the first. This is likely to be due to an increase in *de novo* synthesis of dopamine which is then preferentially released instead of stored [^{3}H]dopamine (Herdon *et al.*, 1985). We suggest that the further reduction in S2 by these two antipsychotics is due to an increase in dopamine synthesis which is an acute effect only. The therapeutic effect of antipsychotics is presumed to be mediated via the blockade of dopamine D2 receptors, although other receptors are likely to be involved. It seems likely that the effects on basal release are mediated via the D2 receptor as haloperidol has a larger effect than clozapine which compares with the *in vivo* receptor occupancy of the two drugs (Farde *et al.*, 1989). However the proposed effects on synthesis of dopamine would appear to be mediated by a system developing tolerance to chronic treatment.

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626P 7-NITROINDAZOLE IS A SHORT-ACTING INHIBITOR OF BRAIN NITRIC OXIDE SYNTHASE

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7-nitro indazole (7-NI) is a potent inhibitor of neuronal nitric oxide synthase (NOS) in vitro and in vivo does not influence blood pressure in the rat (Moore et al., 1993). Thus, 7-NI may be of use in investigating the biological effects of nitric oxide in the central nervous system. We have now studied the time course of inhibition of rat brain NOS following parenteral administration of 7-NI. Male Wistar rats (200-300g) were treated with 7-NI (30 mg/kg) or 6 ml/kg arachis oil (i.p./orally) and killed by cervical dislocation at timed intervals thereafter. NOS activity was determined in striatal and cerebellar homogenates by conversion of [3H]-L-arginine to [3H]-citrulline and expressed as pmol citrulline/mg protein/15 min (Moore et al., 1993). NOS activity was 5.66±0.34 and 5.37±0.24, (n=8) in the striatum and cerebellum of untreated rats. No change in NOS activity was detected in animals treated with arachis oil. Maximal inhibition of striatal and cerebellar NOS was observed 0.5 h following i.p. or oral injection of 7-NI. NOS inhibition declined with time and was not apparent at 24 h (Table).

Table: Effect of 7-NI on striatal and cerebellar NOS activity (n=5, *P<0.05, Dunnett's test, ND=not determined).

Brain region		Time (h)					
		Vehicle	0.5	2	3	4	24
Striatum	i.p.	5.41±0.40	1.00±0.07*	1.36±0.04*	1.70±0.12*	2.70±0.25*	5.51±0.12
	oral	4.38±0.35	2.57±0.15*	3.60±0.38	3.90±0.50	5.32±0.67	ND
Cerebellum	i.p.	6.65±0.40	1.33±0.14*	1.82±0.15*	2.36±0.27*	3.32±0.46*	6.40±0.19
	oral	6.00±0.36	2.31±0.13*	3.90±0.32*	4.23±0.56*	4.91±0.25	ND

In further experiments, rats were treated with 7-NI (30 mg/kg, i.p.) or vehicle every 4h for 20 h and subsequently killed at 24 h. In these animals striatal NOS activity was inhibited by 7-NI pretreatment (2.24±0.35 vs 5.72±0.29 in vehicle injected rats, n=4). In separate experiments, rats were given two injections (6-8 h apart) of either L-NAME (50 mg/kg, i.p.) or 7-NI (60 mg/kg, i.p.) and striatal NOS activity measured 24 h after the first injection. Striatal NOS activity was inhibited by L-NAME (0.47±0.05 vs 5.74±0.90, n=5) but not 7-NI (4.73±0.40 vs 5.03±0.03, n=3).

Thus, 7-NI appears to produce a relatively short-lasting inhibition of brain NOS activity compared to L-NAME. In addition, 7-NI appears to be less effective following oral administration compared to the intraperitoneal route.

GMM is supported by an SERC-CASE award in collaboration with Wellcome Foundation Ltd.

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627P NMDA RELEASES A NITRIC OXIDE-LIKE SUBSTANCE IN RAT STRIATUM: A STUDY USING FAST CYCLIC VOLTAMMETRY

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We recently described a method for the detection of NO (nitric oxide) in aqueous solution using FCV (fast cyclic voltammetry) (Iravani et al., 1993). We have now detected an NO-like signal following pressure ejection of NMDA (N-methyl d-aspartic acid) in slices of rat striatum.

350 µm slices were prepared from albino male Wistar rats (180-250 g) and set up for FCV according to the protocol of Trout & Kruk (1992). They were superfused at a rate of 1 ml/min at 32° C with 95% O_2 and 5% CO_2-saturated Mg^{2+}-free artificial cerebrospinal fluid. 0.1 M NMDA was pressure ejected in the fluid just above the region of the slice containing the carbon fibre electrode at 3-5 psi for 2s, using a micropipette with a tip diameter of 10-20 µm. In conventional FCV a "W" shaped drive waveform with a peak at 1.4 volts is used. In order to minimise the artefacts associated with the sharp change of voltage at +1.4 volts, in these experiments we have used a sinusoidal drive waveform (Millar, in preparation). Addition of 10 µM NO dissolved in deoxygenated phosphate buffered saline pH 7.4, produced two reduction signals (r_1 at -426 ± 74 mV; r_2 at -348 ± 72 mV; (mean ± s.d.; n=11) and an oxidation signal peaking at +1260 ± 58 mV (mean ±s.d.; n=11).

Pressure ejection of NMDA produced a complex but reproducible voltammetric signal. It included two reduction peaks which occurred at similar voltages to those seen with NO. The oxidation signal was broad, with a main peak at +600 mV (the dopamine oxidation potential), and a smaller, wider peak at between +1200 and +1300 mV, corresponding to the oxidation potential of NO. Addition of 100 µM of the NOS-inhibitor, L-NMMA to the bathing solution led to a gradual decline of the signal at +1260 mV. After 50 min L-NMMA exposure the signal had declined to 36 ± 7 % of control (mean ±s.e.m.; n=5, p =0.028, t-test). The results of these experiments indicate that NMDA may release 2 different substances. The large amplitude current at about +600 mV may reflect dopamine release (Iravani & Kruk 1992). The smaller oxidizing current with a peak at about +1260 mV and the double reduction currents indicate the release of NO or an NO-like substance. The experiments showing a reduction of these latter signals by L-NMMA support this suggestion.

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628P IFENPRODIL AND CGS 19755 DISCRIMINATE BETWEEN CORTICAL AND CEREBELLAR NMDA RECEPTORS

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Recent receptor cloning studies have clearly indicated the existence of multiple NMDA receptor subunits in rat brain and it is generally accepted that NMDA receptors exist as hetero-oligomeric subunit assemblies. We have recently demonstrated that native NMDA receptors on rat cultured cortical neurones are different to those on rat cultured cerebellar granule cells with respect to their *agonist* pharmacology (Priestley and Kemp, 1993). In the present study, we have examined NMDA receptor *antagonist* pharmacology on these two cell types and report differences in affinity both for a noncompetitive antagonist, ifenprodil and a competitive antagonist, CGS 19755.

Experiments were performed on whole-cell voltage-clamped (clamp potential = -60mV) foetal rat cortical or neonatal rat cerebellar granule cell cultures (for methodological details see Priestley and Kemp, 1993). Fast perfusion of N-methyl-D-aspartate (NMDA, 100μM) + glycine (10μM), in a magnesium-free artificial cerebrospinal fluid, produced inward current responses. Ifenprodil and CGS 19755 antagonised such responses in a concentration-dependent manner. The resulting inhibition curves were used to derive the concentration of antagonist required to reduce the control agonist response by 50% (expressed as the negative logarithm: pIC_{50}). The pIC_{50} value for the action of ifenprodil at cortical NMDA receptors was 5.76 ± 0.12 (n = 7), whereas at granule cell NMDA receptors the corresponding value was 4.13 ± 1.16 (n = 6, significant difference P<0.0001, unpaired t-test), i.e. the antagonist had ~40-fold lower affinity for the cerebellar receptor . The competitive antagonist, CGS 19755, showed the opposite selectivity, having an ~5-fold higher affinity for granule cell NMDA receptors (pIC_{50} = 5.41 ± 0.08, n = 5) compared to those on cortical neurones (pIC_{50} = 4.77 ± 0.13, n = 4, significant difference P<0.001, t-test).

The present data support our previous suggestion (Priestley and Kemp, 1993) that native NMDA receptors on rat cultured cerebellar granule cells and cortical neurones are different. Ifenprodil has been reported to show selectivity for NMDAR2B subunit-containing NMDA receptors (Williams, 1993), the higher affinity of this compound for the cortical cell receptor suggests that these receptors contain the 2B subunit whereas those on cerebellar granule cells do not. The reverse profile of CGS 19755 implies that the presence of a different subunit, possibly NMDAR2A (see Priestley and Kemp, 1993), is important for the high affinity binding of this antagonist. The different profiles of ifenprodil and CGS 19755 suggest that these antagonists may be of value in identifying other subpopulations of native NMDA receptors.

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Williams, K (1993) Mol Pharmacol. 44, 851-859

629P CONTRASTING EFFECTS OF NON-COMPETITIVE, COMPETITIVE AND GLYCINE-SITE NMDA RECEPTOR ANTAGONISTS ON PREPULSE INHIBITION IN THE RAT

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Prepulse inhibition of the acoustic startle response is attenuated both in schizophrenic patients and in rodents given the non-competitive NMDA receptor antagonist, phencyclidine (PCP), which is psychotomimetic in man. Whilst the effect of PCP in rodents is likely to result from activation of mesocorticolimbic dopamine systems, it is now increasingly evident that different classes of NMDA receptor antagonist modulate dopamine systems in different ways. In the present study, we have examined the ability of the non-competitive NMDA receptor antagonist, MK-801, the competitive NMDA receptor antagonist, CGS 19755 (cis-4-(phosphonomethyl)piperidine-2-carboxylic acid) and the glycine/NMDA receptor antagonist, L-695,902 (7-chloro-4-hydroxy-3-methoxycarbonyl-2-quinolone; Rowley et. al., 1993) to attenuate prepulse inhibition in the rat.

Startle responses in male Wistar rats (290 - 450 g) were recorded 10 min after drug administration using 6-SR-LAB (San Diego Instruments) stabilimeter chambers. Following a 5 min acclimation period to background noise (65 dB), startle responses were recorded to 15 repetitions of 4 different stimuli: i) prepulse (80 dB tone, 5 msec duration), ii) prepulse + pulse (140 dB tone, 40 msec duration beginning 100 msec after onset of the prepulse), iii) pulse alone and iv) a period when no stimulus was presented. Stimuli were presented in a random order with a mean interstimulus interval of 15 sec. Mean startle amplitude ± s.e.mean for each stimulus type was calculated for each subject (n = 12 rats per dose group) and prepulse inhibition calculated as the % reduction in startle responding i.e. 100 x [pulse - (prepulse + pulse)]/pulse (analysed by ANOVA and Dunnett's t test; * = P<0.05 compared to vehicle-treated rats).

Rats pretreated with vehicle showed a mean reduction of 57% in amplitude of the startle response when a 140 dB stimulus (pulse) was preceded by a 80 dB prepulse (pulse alone = 557 ± 21; prepulse + pulse = 227 ± 18*). Pretreatment with the non competitive NMDA receptor antagonist, MK-801, significantly attenuated prepulse inhibition in the rat (% inhibition: veh = 57 ± 4; 0.1 mg/kg = 14 ± 4*; 0.2 mg/kg = 2 ± 2*, 0.4 mg/kg = 2 ± 2*) at doses which did not significantly alter startle responses to the pulse alone. In contrast, pretreatment with the competitive NMDA receptor antagonist, CGS 19755, failed to alter prepulse inhibition (% inhibition: veh = 53 ± 6; 1 mg/kg = 50 ± 6; 3 mg/kg = 49 ± 4; 10 mg/kg = 47 ± 7). A similar lack of effect was also seen in rats treated with the glycine/NMDA receptor antagonist, L-695,902 (% inhibition: veh = 60 ± 5; 3 mg/kg = 58 ± 3; 10 mg/kg = 56 ± 4; 30 mg/kg = 61 ± 5) although a significant reduction in startle responses to the pulse alone was recorded at the highest dose (veh = 515 ± 94; 30 mg/kg = 130 ± 20*).

In conclusion, the marked disruption of prepulse inhibition following MK-801 injection is consistent with prevous studies showing that MK-801 will activate mesocorticolimbic dopamine systems. In contrast, the lack of effect of CGS 19755 and L-695,902 provides further evidence of the contrasting behavioural profiles of different classes of NMDA receptor antagonist.

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630P THE DESGLYCINE METABOLITE OF REMACEMIDE (FPL 12495) INHIBITS THE RELEASE OF GLUTAMATE FROM MOUSE CORTICAL SLICES

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The desglycine derivative (FPL 12495) is the main pharmacologically active metabolite of remacemide and is more potent than the parent compound against maximal electro-shock (MES) induced seizures in rats and N-methyl-DL-aspartate-induced convulsions in mice (Palmer *et al,* 1992). This study investigated the effect of the desglycine metabolite on the potassium and veratridine-stimulated release of glutamate from mouse cortical slices.

Coronal slices (400μm) were prepared from the cortex of male or female adult BALB/c mice (25-30g) using a McIlwain tissue chopper and then perfused with gassed (95% O_2 / 5% CO_2) artificial cerebrospinal fluid at 37°C. Two-minute aliquots of perfusate were collected and assayed for glutamate by HPLC with fluorometric detection. Transmitter release was elicited with three two-minute pulses of potassium (60mM) at samples 4, 14 and 24 or two one-minute pulses of veratridine (20μM) during the collection of samples 4 and 14. The drug was perfused between samples 7 and 17. The results are presented as the mean stimulated release per two-minute sample as a percentage of basal release. The basal release of glutamate was between 2 -5 pmoles/mg tissue/2 minutes.

Potassium (60mM) and veratridine (20μM) both produced significant increases in the release of glutamate (800% and 700% respectively). The second and third pulses of potassium resulted in an average glutamate release of 88±6% and 87±4% respectively of the first pulse, while the second pulse of veratridine resulted in an average glutamate release of 68±6% of the first pulse. The potassium-stimulated release of glutamate was inhibited significantly by FPL 12495 at concentrations of 100μM (46±3%, P <0.001, n=7) and 200μM (28±6%, P <0.01, n=5). Veratridine-stimulated release of glutamate was significantly inhibited by concentrations of 25μM (31±11%, P <0.001, n=6), 50μM (25±7%, P <0.001, n=8) and 100μM (30±6%, P <0.01, n=8).

Radioligand binding studies have indicated that remacemide has a low affinity for both the glycine and the ion channel sites on the N-methyl-D-aspartate subtype of glutamate receptor, but the desglycine metabolite is considerably more potent in displacing dizocilpine binding than is the parent compound (IC_{50} 0.48 and 68μM respectively, Palmer, *et al.,* 1992). Such an effect may account for its anticonvulsant action. However, glutamate is involved in the pathogenesis of epilepsy and the anticonvulsant effect of remacemide could be due, in part, to the inhibitory effect of the desglycine metabolite on the release of glutamate.

Acknowlegement We should like to thank Dr J.B. Farmer, Fison's Pharmaceuticals Division, for the gift of FPL 12495.

Palmer, G.C., Murray, R.J., Wilson, C.M.T. *et al.,* (1992) *Epilepsy Res.* 12, 9-20.

631P [^{3}H]GLYCINE BINDING IN FOREBRAIN AND CEREBELLUM HOMOGENATES TO NMDA AND NON-NMDA RECEPTORS

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Radioligand binding studies in rat brain and spinal cord have demonstrated that [^{3}H]glycine recognises two sites here called $glycine_A$ and $glycine_B$. $Glycine_A$ sites are located on the strychnine-sensitive chloride channels located in spinal cord and medulla but are absent in forebrain (Young and Snyder, 1977). The strychnine-insensitive $glycine_B$ sites are located primarily in forebrain and are associated with the glutaminergic ionotrophic NMDA receptors (Johnson and Ascher, 1987). We have previously demonstrated that the glutamate recognition site on cerebellar NMDA receptors is pharmacologically distinct from the NMDA receptors in forebrain. Also the modulation by glycine ligands of glutamate antagonist binding to NMDA receptors is more pronounced in cerebellum as compared to forebrain (Widdowson et al., 1994). We therefore examined whether we could detect a difference in the pharmacology of the glycine site of cerebellar NMDA receptors, as compared to forebrain. In addition we studied the modulation of [^{3}H]glycine binding by glutamate ligands in the two brain regions. The characteristics of [^{3}H]glycine binding was examined in well-washed, Triton X-100 treated forebrain and cerebellar membranes prepared from adult rat brain. Binding was performed in Tris/acetate buffer (pH 7.2) at 20 °C for 12 min. and bound separated from free by rapid centrifugation. [^{3}H]Glycine binding in forebrain recognised a single population of sites with high affinity (Kd = 112 ± 31 nM; Bmax = 2.31 ± 0.25 pmol/mg protein; mean ± S.E.M.). The pharmacological profile of this site was similar to the glycine recognition site of NMDA receptors, with a high affinity for D-serine (Ki = 0.32 ± 0.14 μM), D-cycloserine (Ki = 4.8 ± 0.2 μM), DL-HA966 (Ki = 24 ± 8 μM), 5,7-dichlorokynurenic acid (Ki = 0.16 ± 0.01 μM) and L-689,560 (Ki = 0.012 ± 0.001 μM). In contrast, [^{3}H]glycine binding in cerebellum recognised two sites with approximately ten-fold difference in affinity (Kd_1 = 64 ± 34 nM; $Bmax_1$ = 3.5 ± 1.3 pmol/mg protein; Kd_2 = 619 ± 194 nM, $Bmax_2$ = 18.7 ± 5.7 pmol/mg protein). The pharmacological profile of the high affinity site was similar to that of the single binding site in forebrain, suggesting that this is the glycine regonition site of the cerebellar NMDA receptor. The lower affinity site did not recognise any of the glycine antagonists (L-689,560, 5,7-dichlorokynurenic acid, kynurenic acid: Ki's >1000 μM) and the affinity for glycine agonists was much lower (D-serine; Ki = 1965 ± 1274 μM; D-cycloserine Ki = 539 ± 122 μM). Neither glutamate nor NMDA altered [^{3}H]glycine binding in either forebrain or cerebellum up to 100 μM. The glutamate antagonists, CGS19755 was able to reduce [^{3}H]glycine binding to the high affinity site 1 in both forebrain and cerebellum, whereas the antagonist CPP inhibited [^{3}H]glycine binding to site 1 only in the cerebellum. In conclusion although the pharmacology of the glycine site appears similar in forebrain and cerebellum, the modulation by CPP is different in the two brain areas. We have also provided evidence that [^{3}H]glycine is able to recognise a site in cerebellum which appears unrelated to $glycine_B$ site (NMDA receptor) but may be related to the $glycine\ site_A$.

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632P SYSTEMICALLY ADMINSTERED KAINATE NEUROTOXICITY CAN BE BLOCKED BY BOTH NMDA AND NON-NMDA ANTAGONISTS

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Systemic kainate administration at a dose ≥10mg.kg^{-1} results in neuronal damage in the hippocampus, which is reflected in an elevation in the B_{max} of peripheral benzodiazepine receptors (PBRs) (MacGregor and Stone, 1993). The mechanism of the kainate induced damage may involve the release of presynaptic glutamate (Ferkany *et al.*, 1982) which can act postsynaptically to activate NMDA receptors. There is increasing evidence for the involvement of NMDA receptors in kainate mediated toxicity (Fariello *et al*, 1989). The non-NMDA receptor antagonists CNQX and GYKI 52466 are able to cross the blood-brain barrier (Moncada *et al.*, 1991). GYKI 52466 has been shown to be a specific antagonist at the AMPA receptor *in vitro*, although it prevents kainate not AMPA toxicity *in vivo* (Moncada *et al.*, 1991). Here we study the effects of 2 types of NMDA receptor antagonists (competitive (CPP ((±)-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid)) and non-competitive (dizocilpine (MK801))), the non- NMDA antagonist GYKI 52466, and L-kynurenine (the precursor for kynurenic acid) on the kainate induced elevation in PBR ligand binding ([^{3}H] PK11195).

Male Wistar rats (180-220g) were injected with 0.2mg.kg^{-1} clonazepam, 10mg.kg^{-1} kainate and either drug or vehicle. MK801, CPP and L-kynurenine were all administered with kainate at time (t_0), and GYKI 52466 (30mg.kg^{-1}) was administered at t_{-15} min, t_0 and t_{+15} min (Moncada *et al.*, 1991). The animals were killed on the seventh day after injection and the hippocampi removed and a mitochondrial P_2 membrane fraction prepared as in MacGregor & Stone (1993). The membranes were incubated for 1 hour on ice with 1.75nM [^{3}H]PK11195. The incubation was terminated by vacuum filtration and DPM counted by scintillation spectrometry.

The kainate administration resulted in an elevation of [^{3}H]PK11195 binding from 70.74 ± 21.37 (n=3) to 234.16 ± 15.43 fmol.mg protein^{-1} (331.01 ± 21.81% control (n=5), mean ± SEM, p≤0.001 Student's t-test). This increase in binding was significantly reduced in a dose dependent manner by MK801 (maximal protection obtained at 3mg.kg^{-1}, (83.60 ± 11.59% control (n=4) p≤0.001)), kynurenine (maximal protection obtained at 75mg.kg^{-1}, (156.01 ± 65.53% control (n=4) p≤0.001)) and CPP (maximal protection obtained at 30mg.kg^{-1}, (134.70 ± 63.42% control (n=4) p≤0.05)). GYKI 52466 was also able to antagonise the kainate induced effect (143.59 ± 34.24% control, (n=4) p≤0.05).

The results indicate that kainate neurotoxicity has both NMDA and non-NMDA components, and support the hypothesis that kainate is able to release glutamate, which can subsequently act on glutamate receptor subtypes to produce neuronal death.

This work was funded by a Glasgow University scholarship (DGM).

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633P NBQX PRETREATMENT MODIFIES MK-801-INDUCED CHANGES IN LOCAL CEREBRAL GLUCOSE USE IN THE POSTERIOR CINGULATE CORTEX

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NMDA receptor antagonists induce a range of neurochemical and morphological alterations within the limbic system, particularly within the posterior cingulate cortex. These alterations include marked increases in glucose utilisation, heat shock protein deposition, immediate early gene expression and neuronal swelling. We have examined if administration of the AMPA receptor antagonist NBQX (Browne & McCulloch, 1994) alters the pattern of glucose use seen with the administration of MK-801 (Kurumaji et al., 1989).

The experiments were carried out on 16 adult male Sprague Dawley rats (290-415g). The rats were fully conscious and were restrained using a loose fitting plaster cast around the pelvis and lower abdomen. NBQX (7mg/kg, i.v.) was administered 2 min prior to the administration of MK-801 (0.2mg/kg, i.v.). The vehicle for the NBQX was 5.5% glucose in saline and that for the MK-801 was 0.9% saline. The measurements of local cerebral glucose utilisation were initiated 5 min after the administration of saline or MK-801 and were carried out as described previously (Sokoloff et al., 1977). Statistical analysis was carried out by ANOVA. Data are expressed as mean ± s.e. mean, n=4 in each group. **P<0.01 (from vehicle-treated), ††P<0.01 (from MK-801-treated).

Table 1 summarises some of the alterations in glucose utilisation. MK-801 (0.2mg/kg) increased glucose use in some components of the limbic system. In the posterior cingulate gyrus, MK-801 caused a marked increase in local cerebral glucose use. NBQX (7mg/kg) had no effect on glucose use in any area examined. NBQX administration prior to MK-801 attenuated the increase in glucose use in the posterior cingulate cortex.

TABLE 1 Local Cerebral Glucose Utilisation (μmol 100g^{-1} min^{-1}) (n=4 in each group)

Structure	Vehicle	MK-801 (0.2mg/kg)	NBQX (7mg/kg)	NBQX + MK-801
Posterior Cingulate Cortex	67 ± 2	101 ± 9**	62 ± 4	69 ± 3††
Hippocampus (Molecular Layer)	56 ± 3	73 ± 5	54 ± 5	66 ± 8
Mamillary Body	81 ± 4	117 ± 12**	73 ± 4	85 ± 3
Sensory Motor Cortex (Layer IV)	88 ± 7	55 ± 3**	85 ± 10	50 ± 2

The attenuation of the hypermetabolism in the posterior cingulate cortex indicates that there may be some interaction *in vivo* between NBQX and MK-801. This combination therapy may be useful in counteracting the potential adverse effects of the non-competitive NMDA receptor antagonists.

TRP is supported by an MRC/CASE studentship.

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634P NOVEL AGONISTS AND ANTAGONISTS OF RAT FOREBRAIN METABOTROPIC GLUTAMATE RECEPTORS NEGATIVELY COUPLED TO ADENYLYL CYCLASE

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Seven metabotropic glutamate receptors (mGluR) have been identified (see Schoepp and Conn, 1993). These have been classified into three groups according to their agonist profile, sequence homology and effector coupling (Tanabe *et al.*, 1993). These groups comprise: (i) mGluR1/mGluR5, which are coupled to phospholipase C and have low affinity for L-2-amino-4-phosphono-butyric acid (L-AP4) but are stimulated by (2S,3S,4S)-α-(carboxycyclopropyl)-glycine (L-CCG-1), (ii) mGluR2/mGluR3, which are negatively coupled to adenylyl cyclase and have low affinity for L-AP4 but are potently activated by L-CCG-1, (iii) mGluR4/mGluR6/mGluR7, which are also negatively coupled to adenylyl cyclase, but are highly sensitive to stimulation by L-AP4.

Novel compounds were investigated for agonist or antagonist activity at mGluRs negatively coupled to adenylyl cyclase. Adult rat forebrain slices (0.3 x 0.3mm) were preloaded with [^{3}H]adenine and adenylyl cyclase was stimulated with 30μM forskolin in the presence of 10μM L-AP4 or 0.3μM L-CCG-1 (their respective EC_{50} values for inhibition of cyclic AMP production in this experimental system) plus the test compound. Adenosine deaminase was also included to remove any adenosine produced. The [^{3}H]cyclic AMP produced was isolated by ion exchange chromatography and estimated by liquid scintillation counting. Results were expressed as a percentage of stimulation of cyclic AMP above basal levels.

mGluR agonists: (2S,3S,4S)-α-(carboxycyclopropyl)-α-alanine (MCCG) exhibited weak agonist activity, with a maximal 40% inhibition of the forskolin stimulation being observed at 1mM. In contrast, (S)-2-amino-2-methyl-4-phosphonobutyric acid (MAP4) was a reasonably potent agonist with an EC_{50} value of 0.1μM.

mGluR antagonists: (RS)-α-methyl-3-carboxymethylphenylglycine (αM3CMPG) antagonised the inhibitory effects of both L-AP4 and L-CCG-1 on forskolin-stimulated cyclic AMP formation, with IC_{50} values of 1μM and 0.2μM respectively. (S)-α-methyl-3-carboxy-4-hydroxy-phenylglycine (αM3C4HPG) was also an effective antagonist of both agonists, with IC_{50} values of 80nM versus L-AP4 and 7nM against L-CCG-1. Both αM3CMPG and αM3C4HPG are thus potent antagonists of both L-AP4 and L-CCG-1. A third compound, (S)-α-methyl-3-carboxyphenylalanine (αM3CPA), was an effective antagonist of L-AP4 (IC_{50}= 1μM) but was virtually inactive against L-CCG-1 (IC_{50} > 0.1mM). These data therefore suggest that mixed populations of mGluRs negatively coupled to adenylyl cyclase are present in rat forebrain slices and that these may be discriminated pharmacologically.

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635P THE EFFECT OF NATRIURETIC PEPTIDES ON SECOND MESSENGER GENERATION IN THE ADULT GUINEA-PIG CEREBELLUM

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The generation of cyclic GMP appears to be mediated by two classes of guanylyl cyclase. The soluble guanylyl cyclase class is activated by nitric oxide, while the particulate forms appear to be involved in signal transduction at the plasma membrane level. Two natriuretic peptide receptor classes which couple to particulate guanylyl cyclase activities (ANP_A and ANP_B) may be defined by the rank order of potency of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and natriuretic peptide type C (CNP) (Maack, 1992). A third class of natriuretic peptide receptor with an as-yet undefined signal transduction mechanism, the clearance receptor, has been also been recognised. The cerebellum of the guinea-pig is a region which we have recently characterized with respect to activation of the soluble guanylyl cyclase activity (Hernández *et al.* 1993; 1994), and which also expresses a high density of ANP binding sites (Quirion *et al.*, 1986). The purpose of the present study was to investigate the influence of natriuretic peptides on second messenger generation in guinea-pig cerebellum and to define which subtypes of natriuretic peptide receptor were present.

Generation of [^{3}H]-cyclic AMP, [^{3}H]-cyclic GMP and [^{3}H]-inositol phosphates was evaluated in guinea-pig cerebellar slices using radio-labelled precursors as previously described (Hernández *et al.*, 1993).

In guinea-pig cerebellar slices examined in the presence of 0.5 mM 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor, ANP induced [^{3}H]-cyclic GMP accumulation in a time-dependent manner, with a maximal response at 5 min, maintained for at least a further 15 min. At 5 min, ANP and BNP evoked concentration-dependent increases in [^{3}H]-cyclic GMP accumulation (EC_{50}=20 ± 8 nM, n=5; and 169 ± 41 nM, n=3). The responses to 1 μM ANP and BNP were 273 ± 16 % (n=7, P<0.01) and 208 ± 10 % of control responses (n=3, P<0.01), respectively. CNP significantly stimulated [^{3}H]cyclic GMP production only at concentrations above 1 μM. Thiorphan, an inhibitor of endopeptidase 24-11, failed to alter EC_{50} values or maximal responses to the three natriuretic peptides.

A 10 min incubation with the natriuretic peptides (all at 1 μM) did not alter the [^{3}H]-cyclic AMP accumulation following 10 min of pre-treatment with 10 μM forskolin (ANP 104 ± 8 % control; BNP 105 ± 5 %; CNP 107 ± 7 %, n=3). A 45 min incubation with the natriuretic peptides failed to significantly stimulate [^{3}H]-inositol phosphates accumulation (ANP 89 ± 19 % control; BNP 82 ± 22 %; CNP 122 ± 15 %, n=3).

These data are consistent with the presence of ANP_A receptors in guinea-pig cerebellar slices coupled to stimulation of guanylyl cyclase activity. We do not find any evidence for the presence of functional ANP_B receptors, or for coupling of natriuretic peptide receptors to either cyclic AMP generation or phosphoinositide turnover.

The financial support of the Medical Research Council, NATO and the Wellcome Trust is gratefully acknowledged.

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636P EFFECT OF THE N-GLUCURONIDE CONJUGATE OF GR117289 AT ANGIOTENSIN AT_1 AND AT_2 RECEPTORS IN THE RABBIT AORTA AND BOVINE CEREBELLUM

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Intravenous administration (1mg kg^{-1}) of the selective and specific angiotensin AT_1 receptor antagonist GR117289 (Robertson et al., 1992) results in a significant amount (~3μM) of the N-glucuronide conjugate of GR117289 (N-Glu-GR117289; [(3-bromo-2-(2-(1-(1-deoxy-1-β-D-glucopyranuronosyl)-2H-tetrazol-5-yl)-phenyl)-benzofuran-5-yl-methyl)-2-butyl-5-chloro-3H-imidazole-4-carboxylic acid]) appearing in the plasma in man (unpublished observations). We have synthesised N-Glu-GR117289 and evaluated it for antagonist activity or binding affinity at angiotensin AT_1 and AT_2 receptors in the rabbit aorta and bovine cerebellum.

Thoracic aortae were removed from male, New Zealand White rabbits and suspended in Krebs' solution containing ascorbic acid (100μM) and indomethacin (30μM) and gassed with 95% O_2 and 5% CO_2 at 37°C. Angiotensin II (AII) was used to contract the tissue in a concentration-related manner and administration was repeated until reproducible responses to AII were obtained and concentration-effect curves differed by <2-fold. N-Glu-GR117289 (10μM) was incubated, with separate tissues for 15, 60 and 120min after which contractile responses to AII were re-established. Subsequently, the effect of N-Glu-GR117289 (0.1, 1.0 and 10μM) on AII contractile responses was determined after 2h incubation. In a final series of experiments, the effect of co-incubation of N-Glu-GR117289 with GR117289 on the blockade exerted by GR117289 in the aorta was established. In these experiments, a constant concentration of GR117289 (1nM) was used which was incubated (2h), with one of three concentrations of N-Glu-GR117289 (0.1μM, 1μM or 10μM). The angiotensin AT_2 ligand binding activity of N-Glu-GR117289 was determined in bovine cerebellum homogenates using a commercially available kit (NENQuest, DuPont).

N-Glu-GR117289 (10μM) inhibited the contractile effects of AII in the rabbit aorta and produced parallel, time-related displacements in AII concentration-effect curves with no suppression of the maximum response. The effect of N-Glu-GR117289 was slow to reach equilibrium (≥2h) and N-Glu-GR117289 was a weak antagonist of AII (pA_2 = 7.3 ± 0.16; slope = 0.80 ± 0.03 , n=4). Co-incubation of N-Glu-GR117289 with GR117289 resulted in a concentration-related reversal of the suppression of the maximum AII contractile response, which was seen with GR117289 alone. Indeed, when GR117289 was co-incubated with the highest concentration of N-Glu-GR117289 (10μM), no suppression was observed and a parallel displacement of the AII concentration-effect curve occurred, similar to that produced with N-Glu-GR117289 (10μM) alone (concentration-ratios: 64 [33-123] and 68 [28-167], respectively). N-Glu-GR117289 (10μM) had no affinity for angiotensin AT_2 ligand binding sites (n=4).

Thus, unlike GR117289, N-Glu-GR117289 is a competitive, *surmountable* antagonist of AII at angiotensin AT_1 receptors being ~300-fold less potent than GR117289. N-Glu-GR117289 may contribute to the pharmacological effect of GR117289 in man as it occurs in the plasma after intravenous administration, in concentrations 100-fold greater than its pA_2 for angiotensin receptors.

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637P CIRCULATING IL-6 IS TRANSPORTED INTO THE BRAIN VIA A SATURABLE TRANSPORT MECHANISM IN THE RAT

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There is evidence to suggest that some of the wide ranging effects of circulating cytokines are initiated in the CNS. However the ability of these peptide to cross the blood brain barrier (BBB) from the periphery has been debatable. Banks et al (1991) and more recently Gutierrez et al (1993) showed that radiolabelled IL-1 and TNF-α can enter the brain of mice via an active transport mechanism, although the rate of transport is low. Since circulating concentrations of IL-1 are usually low the biological relevance of this system is uncertain. In contrast plasma IL-6 concentrations increase dramatically, for instance during fever. In the present study we have therefore investigated the ability of IL-6 to enter the brain following systemic injection in rats.

Male, Sprague-Dawley, rats weighing 150-200g were anaesthetised (60mg/kg sodium pentobarbitone, ip) and the femoral artery cannulated. The animals were injected intravenously with 2μCi (128ng) of ^{125}I hrIL-6 which in some animals was co-injected with 10μg of unlabelled hrIL-6. For comparison, 2μCi (159ng) of rat albumin (RA) was administered to control animals. Another group of animals received 0.5μCi (32ng)^{125}I IL-6 or 0.5μCi (39.7ng)^{125}I RA into the lateral ventricle of the brain via an indwelling cannula. Blood samples were collected from the femoral artery immediately before the injection, then at 1,2,5 and 10 min after. The animals were then killed and perfused with 0.9% saline through the left ventricle of the heart to remove blood from the tissues, and the brain removed for measurement of radioactivity. In some experiments the CSF, was collected from the animals via the cisterna magna prior to perfusion. Results are presented as area under the curve over a 10 min period.

Brains from animals injected intravenously with ^{125}I IL-6 showed significantly higher radioactivity than those from animals that had received the same amount of iodinated RA. The amount of IL-6 that entered the brain during the 10 min period was equivalent to 277±3 pg/g tissue over 10 min or 0.02% of the original dose, compared to 6±1 pg/g/tissue of albumin over 10 min in RA injected animals, which is 0.0035% of the original dose ($P<0.001$). Similarly, a much higher radioactivity was detected in the CSF of animals that had been injected iv with ^{125}I IL-6 than those treated with ^{125}I RA. The uptake of radioiodinated IL-6 by the brain was significantly inhibited (121±18 pg/g tissue over 10 min or 0.0095% of the original dose), when co-injected with 10μg of unlabelled IL-6. There was no significant difference between the ability of radiolabelled IL-6 or albumin to cross over from the brain to the periphery following icv injection of 0.5μCi.

These results indicate that IL-6 is able to cross the BBB and enter the brain from the periphery, probably via a saturable active transport mechanism. These data support the concept of direct blood to brain transport of cytokines to affect brain function.

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638P *MYO*-INOSITOL HEXAKISPHOSPHATE-STIMULATED CALCIUM MOBILISATION IN RAT NEOCORTICAL SYNAPTOSOMES

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Although the putative neurotransmitter *myo*-inositol hexakisphosphate (IP_6; phytate) is known to accumulate in discrete regions of the rat brain and to exert central effects (Vallejo *et al,*. 1987), the mechanisms by which this sugar phosphate affects neuronal activity are not clear. Previous studies employing indirect measurements of changes in intracellular Ca^{2+} ($[Ca^{2+}_f]_i$) in cerebellar neurones and pituitary cells suggest that IP_6 stimulates an increase in $[Ca^{2+}_f]_i$ (Law *et al.*, 1988; Nicoletti *et al.*, 1989; Sortino *et al.*, 1989). In the present study we used a direct method to measure the effects of IP_6 on $[Ca^{2+}_f]_i$ in rat neocortical synaptosomes.

P2 synaptosomal fractions were prepared from the neocortices of male Wistar rats as described by Gray & Whittaker (1962) and loaded with Fura-2 by the method of Ashley *et al.* (1984). $[Ca^{2+}_f]_i$ was calculated from the Fura-2 fluorescence of synaptosomes incubated at 37°C with stirring in a modified Krebs Henseleit buffer containing 1mM $CaCl_2$ and 0.6mM $MgCl_2$.

Basal $[Ca^{2+}_f]_i$ was 171±27nM (mean ±se mean) (n=12). IP_6 and KCl-stimulated increases in $[Ca^{2+}_f]_i$ above basal, determined 2 min. after drug additions, are shown below. Whereas pretreatment of the synaptosomes with 30μM verapamil significantly reduced the KCl-stimulated response to 24±38nM (n=5; $P<0.05$, paired *t* test), it had no effect on the response to IP_6 (128±42nM; n=7).

Table 1

Addition	10μM-IP_6	30μM-IP_6	100μM-IP_6	300μM-IP_6	40mM-KCl
Increase in $[Ca^{2+}_f]_i$ (nM)±se mean (n)	86±10 (5)	117±34 (5)	134±18 (11)	160±50 (5)	162±25 (9)

These data indicate that the action of IP_6 in rat neocortical synaptosomes involves increased $[Ca^{2+}_f]_i$ and that this effect is not due to mobilisation of extracellular Ca^{2+} via activation of verapamil-sensitive calcium channels.

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639P NORADRENALINE REUPTAKE SITES LABELLED BY [^{3}H]NISOXETINE: LACK OF EFFECT OF ANTIDEPRESSANT TREATMENTS

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Attempts to label noradrenaline (NA) reuptake sites have not met with the same success as labelling of reuptake sites for 5-hydroxytryptamine. [3H]Desipramine and [3H]mazindol have been used as radioligands for NA reuptake sites. However, neither has proved ideal as they both label sites in addition to the NA reuptake site (Javitch et al., 1984; Backstrom et al., 1989). More recently, nisoxetine, a potent and selective NA reuptake inhibitor, has been demonstrated when tritiated to be suitable as a radioligand for labelling NA uptake sites (Tejani-Butt et al., 1990; Tejani-Butt, 1992; Cheetham et al., 1994). Using this radioligand we have examined the effects of acute and chronic administration of several antidepressant drugs and electroconvulsive shock (ECS) on NA reuptake sites in rat frontal cortex.

Male CD rats (100-120g) were administered antidepressant drugs (Table 1) or saline orally once daily for 1 or 21 days or anaesthetised with halothane and given ECS (200V, 2s) either once or 5 times over a 10 day period. Frontal cortices were removed 24h after the final treatment. Freshly prepared membranes (7.5mg wet weight tissue/tube) were incubated with [3H]nisoxetine at 8 concentrations ranging from 0.1-12.8nM for 4h at 0°C. Non-specific binding was defined by mazindol (1μM). Binding parameters (Kd and Bmax) were determined using non-linear least-squares fitting (LIGAND).

Table 1 Effect of repeated administration of antidepressant drugs and ECS on [3H]nisoxetine binding.

	Bmax	Kd		Bmax	Kd		Bmax	Kd
Control n = 29	118 ± 3	0.88 ± 0.03	Fluoxetine (10)	109 ± 6	0.98 ± 0.05	Tranylcypromine (5)	110 ± 6	0.94 ± 0.03
Desipramine (10)	124 ± 6	1.81 ± 0.27**	Bupropion (10)	113 ± 6	0.97 ± 0.04	Halothane control	117 ± 5	1.03 ± 0.03
Amitriptyline (10)	137 ± 6	0.68 ± 0.03	Nomifensine (5)	119 ± 6	0.96 ± 0.05	Halothane ECS	119 ± 5	1.06 ± 0.05

Bmax (fmol/mg protein) and Kd (nM) values ± s.e.mean; n=9-10. Dose mg/kg in parentheses. **p<0.01 from control (Dunnett's test).

[3H]Nisoxetine binding was of high affinity and fitted well to a single site binding model. Kd and Bmax values obtained compare well with previous reports (Tejani-Butt et al., 1990; Tejani-Butt, 1992). Neither acute nor chronic administration of a wide variety of antidepressant drugs or ECS altered the number of [3H]nisoxetine binding sites (table 1; acute data not shown). In addition, no differences in binding affinity were observed, except following repeated desipramine treatment when a significant decrease (increased Kd) was found. One possible explanation for this difference is the presence of residual drug in the brain 24 h after dosing. Thus, repeated antidepressant drug or ECS administration does not alter the number of NA reuptake sites labelled with [3H]nisoxetine. These findings support and extend those of Bauer and Tejani-Butt (1992).

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640P CCK$_A$ RECEPTORS MEDIATE A DEPOLARIZATION OF THE ISOLATED CERVICAL VAGUS NERVE OF THE RAT

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The presence of cholecystokinin (CCK) binding sites of the CCK$_A$ and CCK$_B$ types has been demonstrated in the rat cervical vagus nerve using autoradiographic techniques (Mercer & Lawrence, 1992). Using similar methods, the coupling of these sites to G proteins has also been inferred (Mercer et al., 1993). We have investigated the effects of CCK agonists on membrane potential in isolated preparations of the rat cervical vagus nerve in an attempt to obtain direct evidence of a functional role of these binding sites.

Extracellular recording of depolarization of the cervical vagus nerve was carried out using a "grease-gap" technique (Rhodes et al., 1992). Male, Sprague Dawley rats (180-220 g) were killed with a blow to the head and cervical dislocation. The vagus nerves were placed in two-chamber tissue baths, one chamber being perfused (3 ml min^{-1}) with Krebs' solution (2.5 mM $CaCl_2$, at 27°C, gassed with 5 % CO_2 in O_2) to which drugs were added. Tissues were exposed in a non-cumulative manner to sulphated CCK octapeptide (CCK-8, 0.3 to 100 nM) or pentagastrin (10 nM to 1 μM, n=6) using 3 min agonist contact times and 10-15 min washout periods. Tissues were exposed to one agonist only. In eight preparations CCK-8 was washed from the tissues after the initial concentration response curve (CRC) and the tissues equilibrated (45 min) with the CCK$_A$-selective antagonist devazepide (0.3 nM). A second CRC to CCK-8 was then constructed. In control preparations (n=6) two CRCs to CCK-8 were obtained in the absence of antagonist.

In the presence of CCK-8, concentration-related depolarizations were observed, the maximum response being 97.8 (80.2 to 115.4) μV and the agonist EC_{50} value was 2.63 (1.95 to 3.63) nM, n=18. Depolarizations were well maintained with no indication of after-hyperpolarization. Concentration-response curves to CCK-8 were displaced to the right in a parallel manner in the presence of devazepide (0.3 nM, geometric mean concentration-ratio 7.1) with no evidence of depression in the maximum response to the agonist (mean maximum response in the presence of devazepide was 84[65 to 110]% of the first CRC maximum, n=8). The mean apparent pA_2 value for devazepide was 10.3 (9.8 to 10.7), n=8. In control preparations there was little evidence of reduced sensitivity to agonist in the second CRC (the apparent geometric mean agonist concentration-ratio was 1.76 [0.83 to 3.72] n=4; the mean maximum response to CCK-8 in the second CRC was 73 [59 to 87] % of the first curve maximum). There was no effect of pentagastrin (10 to 100 nM) on the potential difference but at 1 μM pentagastrin evoked a mean depolarization of 50.0 (29.9-70.1) μV, n=6.

The potency of CCK-8 and the apparent pA_2 value for devazepide are consistent with a CCK$_A$ receptor-mediated mechanism of depolarization. In contrast, the CCK$_B$ receptor-selective agonist pentagastrin was active only at high concentrations at which it is known to stimulate CCK$_A$ receptors. Thus these results provide evidence for the presence of functional CCK$_A$ receptors on fibres in the cervical region of the rat vagus nerve.

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641P THE ANXIOLYTIC EFFECTS OF THE CCK$_A$ RECEPTOR ANTAGONIST DEVAZEPIDE IN THE RAT ZERO-MAZE ARE SUPPRESSED BY 5-HT RE-UPTAKE INHIBITION

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Previous studies have shown that the CCK$_A$ receptor antagonist devazepide has an anxiolytic effect on mouse behaviour in the black/white box model (Hendrie et al., 1993). In the present study the zero-maze (Shepherd et al., 1994) which is a modification of the elevated x-maze was used to investigate the effect of devazepide on behaviours associated with anxiety in rats. The study also investigated potential CCK/5-HT interactions by examining the effects of the selective 5-HT uptake inhibitor zimelidine on the devazepide-induced behaviours. Behavioural testing was carried out on male Lister-hooded rats (12/group) as previously described (Shepherd et al., 1994). Drug treatments and behavioural analysis were carried out blind, with drugs administered s.c. 30 min prior to the 5 min test period on the maze. The following behaviours were scored: head dips over the 'open' quadrant platform (HDP) and stretched-attend postures from the 'enclosed' to the 'open' quadrants (SAP) as well as percentage time spent in 'open' quadrants (%TO). To normalise the behavioural scores for parametric analysis, HDP and SAP scores were square root transformed (Shepherd et al., 1994). Diazepam (0.25, 0.5 mg/kg) and the 5-HT$_{2C}$ agonist m-chlorophenylpiperazine (mCPP) (0.5, 1.0 mg/kg) were administered as anxiolytic and anxiogenic standards respectively to validate the model. Diazepam produced dose-dependent anxiolytic effects on HDP (increased, $F_{2,30}$=6.55) and SAP (decreased, $F_{2,30}$=9.29); conversely mCPP produced dose-dependent anxiogenic effects on HDP (decreased, $F_{2,33}$=5.38), SAP (increased, $F_{2,33}$=17.38) and %TO (decreased, $F_{2,33}$=3.89). Devazepide (0.005-1.5 mg/kg) had an anxiolytic effect on both HDP (increased, $F_{7,79}$=3.67) and SAP (decreased, $F_{7,79}$=3.68) but no effect on %TO. Devazepide-induced anxiolysis was not dose dependent - rather it showed a 'bell-shaped' response curve, with the most effective dose being 0.015 mg/kg. In the final experiment, devazepide (0.015 mg/kg) again showed an anxiolytic profile, but, in the presence of zimelidine (3.0, 6.0 mg/kg) this action was dose dependently and significantly attenuated (see table).

Behaviour	Control	Dev	Zim (3.0)	Dev+Zim (3.0)	Zim (6.0)	Dev+Zim (6.0)
HDP	2.00 ± 0.15	3.09 ± 0.24**	1.99 ± 0.26	2.37 ± 0.20#	1.51 ± 0.23	1.63 ± 0.13##
SAP	1.53 ± 0.19	0.67 ± 0.21**	1.61 ± 0.29	1.56 ± 0.19##	1.85 ± 0.20	2.14 ± 0.18##

**p<0.01 vs control; #p<0.05, ##p<0.01 vs Devazepide (Dev). Zim=zimelidine.

These results, in agreement with Hendrie et al. (1993), demonstrate anxiolytic properties of the CCK$_A$ antagonist devazepide. Furthermore, as this anxiolytic profile can be inhibited by the blockade of 5-HT re-uptake sites, the results indicate a functional interaction between CCK and 5-HT in the modulation of anxiety.

MJB is a SERC CASE award student.

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642P EFFECT OF SHORT- AND LONG-TERM LITHIUM TREATMENT ON 4-AMINOPYRIDINE-STIMULATED *IN VIVO* RELEASE OF 5-HT IN RAT HIPPOCAMPUS

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Central 5-HT function in rats is affected by lithium treatment (Price et al., 1990), thus chronic lithium treatment increases K^+-evoked release of 5-HT from hippocampal slices (Friedman and Wang, 1988). In addition 3 day lithium treatment enhances electrically evoked 5-HT release in vivo (Sharp et al., 1991). We have previously shown that different types of potassium channel blocking drugs increase both 5-HT and dopamine release (Zetterström et al., 1991). Others have shown that lithium interacts with K^+-channels (Kato et al., 1991) and this prompted us to investigate the effect of short (3 day) and long (21 day) term treatment with dietary lithium on 5-HT and dopamine release evoked by 4-aminopyridine (4-AP) or TEA in the hippocampus and nucleus accumbens respectively.

Male Sprague-Dawley rats were fed normal chow pellets (control group) or pellets containing 0.1% Li_2CO_3 for 3 or 21 days. Plasma levels in lithium treated rats were measured using atomic absorption spectroscopy and estimated to be 0.52±0.039 mM (n=9) and 0.50±0.027 mM (n=9) for the 3 and 21 day treatment respectively. On experimental days, rats were injected with chloralhydrate (450 mg/kg, i.p.) and kept anaesthetized throughout the experimental period. A dialysis probe was implanted in ventral hippocampus or nucleus accumbens and perfused with artificial cerebrospinal fluid solution containing citalopram (1 µM) for the 5-HT measurements. Monoamines were measured in dialysates using HPLC with electrochemical detection. Following at least three stable baseline measurements, drugs were added via the perfusion medium for the remaining experimental period (120 min). Statistical analyses were made using Student's unpaired t-test.

Basal levels of hippocampal 5-HT (0.043±0.006 pmol/40µl, n=21 rats) and dopamine in nucleus accumbens (0.053±0.005 pmol/40µl, n=14 rats) were unaltered in both short and long term lithium treated rats. Local perfusion with 4-AP (100µM for 120 min) in the hippocampus resulted in increased 5-HT levels in hippocampal dialysates compared to pre-drug values (maximal effect 60 min after onset of administration, 176±9%, n=7). Long term (21 days) lithium treatment significantly ($p>0.05$) enhanced the maximal stimulation of 5-HT efflux in hippocampus dialysates (281±37%, n=7) induced by 4-AP whereas short term lithium treatment did not, (202±13%, n=7). TEA also increased hippocampal 5-HT release, (296±19%, n=6); this effect was not changed by short or long term lithium treatment. Local perfusion with 4-AP (100µl) in nucleus accumbens also enhanced levels of dopamine in dialysates from this brain region (265±32%, n=11), but long term lithium treatment had no effect on this (221±20.7%, n=14).

In summary we have shown that long term lithium treatment enhances the 4-AP-stimulated efflux of 5-HT in the hippocampus but not the efflux of dopamine in the nucleus accumbens. It is suggested, that chronic lithium treatment may alter hippocampal voltage-sensitive K^+-channels which modulate 5-HT release.

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643P CHRONIC TREATMENT WITH PAROXETINE AND FLUOXETINE, BUT NOT DESIPRAMINE, DESENSITISES 5-HT_{2C} RECEPTOR FUNCTION

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Chronic treatment with selective 5-HT reuptake inhibitors (SSRI) are uniquely therapeutic in Obsessive Compulsive Disorder (OCD). It has also been reported that m-chlorophenylpiperazine (mCPP) precipitates OCD in OCD patients, an effect prevented by the 5-HT_1 and 5-HT_2 receptor antagonist metergoline and by chronic SSRI treatment (see Kennett 1993). As mCPP is thought to act as a 5-HT_{2C} receptor agonist (see Kennett 1993) these receptors may be supersensitive in OCD. In the present study the possibility that SSRI's act by desensitising 5-HT_{2C} receptors was assessed using an *in vivo* model of 5-HT_{2C} receptor function, mCPP-induced hypolocomotion (Kennett, 1993).

Male CFY rats (weighing approximately 300g) were held under a 12 h light/dark cycle (lights on 07.00 h) with free access to food and water. Rats were dosed orally with paroxetine, fluoxetine (10 mg/kg), clomipramine (70 mg/kg), desipramine (10 mg/kg) or 1% methyl cellulose vehicle daily for 21 days. 24 h after the last pretreatment dose naive rats were given mCPP 2, 4 or 6 mg/kg i.p. 20 min pretest and placed singly in automated locomotor activity boxes for 10 mins under red light and the number of transits recorded. Brain mCPP levels were determined by HPLC (Suckow 1983) in another group of chronic paroxetine or fluoxetine (10 mg/kg p.o.) treated rats 30 min after injection of mCPP 4 mg/kg i.p. and 24 h after the last SSRI dose. Responses to each dose of mCPP after drug pretreatment were compared to equivalent vehicle pretreated groups by 1 way ANOVA and Dunnett's test and are cited as means ± s.e.m., n=8-19 per group.

mCPP reduced locomotion from 17.4 ± 1.1 transits in saline treated rats to 6.1 ± 1.2 after 4 mg/kg and 4.8 ± 1.6 after 6 mg/kg i.p. (both $p<0.01$). Chronic paroxetine and fluoxetine significantly attenuated the effect of mCPP at 4 and 6 mg/kg i.p., to 14.6 ± 2.3 ($p<0.05$) and 13.2 ± 2.4 ($p<0.05$) respectively after fluoxetine and 16.7 ± 1.8 ($p<0.01$) and 13.2 ± 3.1 ($p<0.05$) after paroxetine pretreatment. Chronic clomipramine tended to prevent the effects of mCPP while desipramine had no effect. However, acute oral administration of fluoxetine (10-100 mg/kg), paroxetine (10-60 mg/kg), or clomipramine (10-70 mg/kg) ,all 1h pretest, did not prevent mCPP-induced hypolocomotion. As chronic fluoxetine and paroxetine did not reduce brain mCPP levels (30 min after 4 mg/kg i.p.) the results suggest that chronic SSRI's, but not desipramine, reduce 5-HT_{2C} receptor responsiveness. This may therefore explain their therapeutic efficacy in OCD.

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644P EFFECTS OF FLUOXETINE, METITEPINE (METHIOTHEPIN) AND GR127935 ON 5-HT EFFLUX IN THE GUINEA-PIG CORTEX *IN VIVO*

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5-HT has been implicated in a number of centrally-mediated disorders including anxiety and depression. 5-HT uptake inhibitors, which increase extracellular 5-HT, have been used extensively as anti-depressants but are only beneficial after chronic dosing. Recently, it has been suggested that antagonists at the 5-HT_{1D} terminal autoreceptor may also enhance 5-HT transmission (Briley & Moret, 1993).

Male Dunkin Hartley guinea pigs (300-400g) were anaesthetized using isofluorane or 50% Vetalar / 50% Rompun (1 ml/kg i.m.) and implanted with a low internal volume 5mm concentric dialysis probe (GFE9 membrane, Gambro) in the frontal cortex (Luparello, 1967 : anterior +15.0mm from ear bar, lateral ±3.5mm, depth 7.0mm from dura, incisor bar -5.0mm). After overnight recovery, the probe was perfused with artificial CSF (125mM NaCl, 2.5mM KCl, 1.18mM $MgCl_2$, 1.26mM $CaCl_2$) at a flow rate of 1 µl/min, 20 minute samples collected into 0.02% L-ascorbic acid and analysed by HPLC with electrochemical detection. Drugs were administered as 3 cumulative infusions through the probe (10, 33 and 100µM) at intervals of 100 minutes and results expressed as a percentage of the mean of 6 basal samples for each animal. Values quoted are mean (for each 100 minute infusion period) ± s.e. mean (n = 4-8). These means were tested in a two-way analysis of variance and t tests performed using the pooled variance (statistical significance was assumed where $p < 0.05$).

Basal 5-HT efflux in guinea pig cortex was 6.3 ± 1.2 fmol/20µl (n = 28). Basal 5-HT levels were increased to 21.5 ± 2.1 fmol/20µl (n = 13) on inclusion of the 5-HT uptake blocker citalopram (1µM) in the CSF. Under these conditions, the 5-HT antagonist (±)-metitepine significantly increased 5-HT efflux at 100µM (128 ± 23 % of basal), but the selective 5-HT_{1D} receptor antagonist GR127935 (N-[4-Methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)[1,1-biphenyl]-4-carboxamide; Skingle *et al.*, 1993) had only a small effect at 100µM which did not reach statistical significance from CSF control. On systemic administration of the monoamine oxidase inhibitor pargyline (75 mg/kg i.p. 16 hours before), basal 5-HT levels (in the absence of citalopram) were further increased to 36.5 ± 3.9 fmol/20µl (n = 36). Under these conditions, 5-HT efflux was significantly enhanced by GR127935 (at 10, 33 and 100µM, reaching a maximum of 183 ± 14 %) and the two isomers of metitepine, the (-) isomer (278 ± 41 %) having a significantly greater effect than the (+) isomer (209 ± 22 %) at 100µM. The 5-HT uptake blocker fluoxetine significantly enhanced 5-HT efflux at 10, 33 and 100µM, reaching a maximum of 366 ± 76 %. These results indicate that blockade of the 5-HT_{1D} terminal autoreceptor enhances extracellular 5-HT more effectively when 5-HT tone is increased but is less pronounced than the effect of fluoxetine.

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645P AN INVESTIGATION OF THE EFFECTS OF ACUTE FLUOXETINE IN A RAT X-MAZE MODEL OF ANXIETY

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When given in the treatment of depression there is evidence that fluoxetine (F), a selective 5-HT reuptake inhibitor, exerts an initial anxiogenic effect which gives way to anxiolysis upon repeated administration (Boyer, McFadden and Feighner, 1991). In the study of Handley and McBlane (1992), an anxiogenic-like effect was observed following acute F in a rat x-maze model, a result that mirrors clinical observations. However, the results were somewhat surprising in that the anxiogenesis was produced by low doses (1.25-10 mg/kg i.p.) of F and there was no effect following the highest dose tested (20 mg/kg i.p.). We have therefore re-examined the effects of acute F in the same model.

Male Sprague Dawley rats (approx. 300g) were dosed (10-11/group) with F (1.25, 2.5, 5, 10 or 20 mg/kg), vehicle (0.9% saline) or, as a positive anxiogenic (Kennett, et al., 1989) control, m-chlorophenylpiperazine (mCPP - 0.6 mg/kg)(all given i.p., 1 ml/kg) 30 min prior to testing in an elevated x-maze test for 5 min as described previously (Blackburn et al., 1993), under white light (785 lux). Results are indicated below (table 1) and demonstrate that, like mCPP, F, at doses of 10 and 20 mg/kg i.p., produced an anxiogenic-like reduction in the time spent on and entries onto the open arm. However, mCPP and the highest dose of F also reduced the number of total crossings, indicating a reduction in locomotor activity.

Table 1: Effect of acute fluoxetine and mCPP on x-maze behaviour in rats

Drug	Dose (mg/kg)	N	Time spent on open arm	Entries onto open arm	Total crossings
vehicle	-	10	67.8 ± 7.6	4.9 ± 0.5	27.4 ± 1.5
mCPP	0.6	11	36.2 ± 5.4**	2.6 ± 0.3*	22.7 ± 1.7*
Fluoxetine	1.25	10	64.9 ± 10.2	4.4 ± 0.4	28.2 ± 1.7
"	2.5	11	62.9 ± 8.9	3.9 ± 0.5	23.9 ± 2.0
"	5	11	81.7 ± 8.4	5.6 ± 0.3	30.4 ± 1.5
"	10	10	37.2 ± 9.2*	2.4 ± 0.6*	19.2 ± 2.8
"	20	10	40.5 ± 9.8*	2.7 ± 0.5*	20.5 ± 2.8*
Kruskal-Wallis H value	-	-	H=21.41, p<0.01	H=29.76, p<0.001	H=18.40, p<0.01

(All values are mean ± s.e.m. of N observations. *p<0.05; **p<0.01 are significantly different from vehicle by Mann-Whitney U test).

These results indicate a lack of anxiogenesis following F at doses of 1.25-5 mg/kg i.p. and an anxiogenic-like effect following doses of 10 and 20 mg/kg i.p. which is in contrast to the bell-shaped dose response relationship seen in the study of Handley and McBlane (1992). Procedural and/or animal strain differences may explain the discrepancy.

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646P ASSESSMENT OF THE DEPENDENCE POTENTIAL OF THE HIGH-EFFICACY 5-HT$_{1A}$ AGONIST, S-14506, IN RATS

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We compared the dependence potential of S-14506, a potent, high-efficacy 5-HT1A agonist putative anxiolytic (Colpaert et al. 1992) with chlordiazepoxide (CDP). Groups of rats (n = 9/10) were treated for 21 days with S-14506 (0.025, 0.25 or 2.5 mg/kg, b.i.d., i.p.) or CDP (10 mg/kg b.i.d. escalating by 2 mg/kg/day to 30 mg/kg b.i.d.; cf. Goudie and Leathley, 1991). Subsequently, withdrawal from both drugs was studied for 7 days by comparing treated groups with vehicle controls. CDP withdrawal caused:- i) Persistent weight loss [in 380 g rats a maximal 5.5 $^{+}_{-}$ 0.5 % drop]; ii) Transient aphagia [a maximal reduction in mean food intake from 19.9 $^{+}_{-}$ 1.1 to 7.4 $^{+}_{-}$ 0.5 gms/day on the second day of withdrawal]; and iii) Transient hyperthermia [a maximal increase of 1.75°C on the second day of withdrawal]. None of these significant withdrawal effects (Tukey tests after ANOVA, $p < 0.05$) were seen after S-14506 withdrawal which caused:- i) Significant persistent weight gain [a maximal 2.5 $^{+}_{-}$ 1.0% increase on day 7 of withdrawal in the 2.5 mg/kg group]; ii) No significant aphagia [a maximal reduction in intake in the 2.5 mg/kg group from 18.7 $^{+}_{-}$ 1.4 to 14.7 $^{+}_{-}$ 1.49 g on the second day of withdrawal]; and iii) No significant hyperthermia [a maximal increase in temperature of 0.5°C on the second day of withdrawal in the 2.5 mg/kg treated group]. Acutely, S-14506 induced significant dose-related hypothermia [a 1.6°C drop at 2.5 mg/kg] to which complete tolerance developed after only one day of treatment, as reported for other effects of 5-HT1A agonists (Kennett et al. 1987; Goudie et al. 1989). The observation of rapid tolerance to S-14506-induced hypothermia, with no hyperthermia on cessation of drug treatment shows that tolerance to this effect of S-14506 is dissociated from a withdrawal effect. More generally, the data suggest that S-14506 is devoid of dependence potential of the benzodiazepine type, in accord with previous studies which have suggested that 5-HT1A ligands do not induce withdrawal signs (e.g. Goudie et al. 1991).

Acknowledgement:- Supported by a grant from I.R.I.S. (Servier), France.

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647P *IN VIVO* ADAPTATIONAL RESPONSES TO THE REPEATED ADMINISTRATION OF SELECTIVE 5-HT$_{1A}$ RECEPTOR LIGANDS

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Previous reports have described the effects of repeated administration of selective 5-HT$_{1A}$ receptor agonists, e.g. 8-OH-DPAT, in models of 5-HT$_{1A}$ receptor function (e.g. Larsson et al., 1990). However, the effects of chronic administration of selective 5-HT$_{1A}$ receptor antagonists have not been reported owing to the lack, until recently, of compounds possessing the appropriate degree of selectivity or functional activity. Here we report a comparison of the effects of chronic administration of 8-OH-DPAT with those of the new selective 5-HT$_{1A}$ receptor antagonist, WAY-100635 (Fletcher et al., 1994), on several in vivo responses mediated by the central 5-HT$_{1A}$ receptor.

Using male Sprague-Dawley rats (initial weight 250-280 g), 8-OH-DPAT was administered subcutaneously from implanted Alzet osmotic minipumps at a rate of 1.5-1.75 mg/kg/day for 14 days. WAY-100635 was administered subcutaneously at a dose of 0.5 mg/kg twice daily for 14 days. On day 15 (24 h withdrawal) animals were tested in the following models: (i) 8-OH-DPAT-induced behavioural syndrome [both by a conventional system of rating following s.c. challenge doses of 8-OH-DPAT, and by determining i.v. ED50 values (with 95% conf. limits) for 8-OH-DPAT to induce the syndrome (Fletcher & Forster, 1989)]; (ii) 8-OH-DPAT-induced hypothermia [body temperature was measured 15-30 min following 0.1 mg/kg s.c. 8-OH-DPAT]; (iii) 8-OH-DPAT-induced hyperphagia [weight of food consumed over 3 h following administration of 8-OH-DPAT (0.1 mg/kg s.c.)].

Chronic administration of 8-OH-DPAT significantly attenuated the syndrome ($P<0.05$) and hypothermic ($P<0.001$) responses to 8-OH-DPAT [ED50 values for 8-OH-DPAT to induce syndrome: chronic vehicle 0.04 (0.03-0.05), chronic 8-OH-DPAT 0.08 (0.07-0.1) mg/kg i.v.; hypothermic response to 0.1 mg/kg s.c. 8-OH-DPAT: chronic vehicle -1.9±0.4, chronic 8-OH-DPAT -0.9±0.1 °C], whereas chronic administration of WAY-100635 had no significant effect. Chronic administration of WAY-100635 had no significant effect on 8-OH-DPAT-induced hyperphagia.

These data indicate that sustained activation of postsynaptic receptors (mediating behavioural syndrome and hypothermic responses) by prolonged administration of 8-OH-DPAT induces an adaptational downregulation, whereas chronic and selective antagonism of the receptor by the selective 5-HT$_{1A}$ receptor antagonist, WAY-100635, does not evoke a significant adaptational response. In a model of somatodendritic 5-HT$_{1A}$ receptor function (hyperphagia) repeated administration of a selective 5-HT$_{1A}$ receptor antagonist had no significant effect. The lack of effect of repeated WAY-100635 administration is consistent with data demonstrating that chronic treatment with this antagonist had no significant effect on the decrease in 5-HT release evoked by somatodendritic 5-HT$_{1A}$ receptor activation (Gurling et al., 1994). These data suggest that the central postsynaptic 5-HT$_{1A}$ receptor can downregulate whereas we were unable to evoke upregulation of functional responses (mediated either by somatodendritic or postsynaptic 5-HT$_{1A}$ receptors) by the repeated administration of a selective 5-HT$_{1A}$ receptor antagonist.

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648P STIMULANT OR DEPRESSANT EFFECT OF THE 5-HT_{1A} AGONIST GEPIRONE ON FOOD INTAKE IN RATS MAY DEPEND ON THE MOTIVATION OF THE ANIMALS TO FEED

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While it is well known that $5HT_{1A}$ agonists, such as 8-OH-DPAT, buspirone and gepirone, increase food intake in non-deprived rats (see Ebenezer, 1992), we have recently reported that these drugs produce decreases in food intake in food-deprived rats (Ebenezer, 1992, Ebenezer and Parmar, 1993) which seem to be related to (i) the dose of drug and (ii) the period of food-deprivation (Ebenezer and Tite, unpublished results). These findings suggest that the feeding responses elicited by $5HT_{1A}$ agonists may depend on the motivation of the animal to feed. In other words $5HT_{1A}$ agonists increase feeding in animals that are not highly motivated to eat, while they decrease feeding in animals that are highly motivated to eat. To test this hypothesis further, the present study was conducted to examine the effects of gepirone on food intake in non-deprived rats in feeding paradigms in which the motivation of the animals to eat was assessed as low or high.

Low Motivational Paradigm. Non-deprived male Wistar rats (n=8, b.wt.280 - 380g) were injected s.c. with either saline or gepirone (1 - 4 mg kg^{-1}) and placed in cages where they had free access to their normal pelleted food and water. Food intake was measured 15, 30, 60 and 120 min after injection. A repeated measures design was used with each rat receiving all treatments in a random manner. 3 to 4 days separated successive trials.

High Motivational Paradigm. A similar experimental proceedure was used except that the non-deprived male Wistar rats (n=10, b.wt.350 - 450g) were given access to a palatable wet mash (The wet mash consisited of 100g powered rat food mixed with 50 ml water and 150 ml full cream evaporated milk). During experimental sessions, the rats were injected s.c. with either saline or gepirone (0.5 - 5 mg kg^{-1}), and food intake measured after 15 min, 30 min, 60 min and 90 min.

Table 1. Effect of gepirone (G) on food-intake (normal pelleted food)

	Mean Food Intake (g) ± s.e. mean			
	0-15 min	0-30 min	0-60 min	0-120 min
Saline	0.7±0.2	1.4±0.4	2.0±0.6	3.6±0.9
G (1 mg/kg^{-1})	1.6±0.2	2.8±0.3	5.0±0.6*	5.7±0.9*
G (2 mg/kg^{-1})	0.7±0.2	2.9±0.1	4.4±0.6*	5.9±0.8*
G (4 mg/kg^{-1})	0.6±0.3	1.4±0.5	4.1±0.7*	7.1±0.7*

*P<0.01

Table 2. Effect of gepirone on palatable food intake

	Mean Food Intake (g) ± s.e. mean			
	0-15 min	0-30 min	0-60 min	0-90 min
Saline	12.9±1.4	15.2±1.5	18.2±1.8	20.1±2.2
G (0.5 mg/kg^{1})	11.6±0.9	14.4±1.3	18.9±1.6	21.3±1.8
G (1 mg/kg^{-1})	9.8±1.2*	15.1±1.4	20.5±2.0	23.1±2.0
G (2 mg/kg^{-1})	6.0±0.7*	10.9±1.1*	17.1±1.6	19.0±1.7
G (5 mg/kg^{-1})	4.5±0.7*	9.2±0.9*	15.6±1.5	18.9±1.8

The results of this study show that while gepirone (1 - 4 mg kg^{-1}) increased food intake in non-deprived rats given access to their normal pelleted food (low motivational paradigm), gepirone (1 - 5 mg kg^{-1}) decreased food intake in non-deprived rats given access to a highly palatable food (high motivational paradigm). These results are consistent with the hypothesis that the stimulant or depressant effects of $5HT_{1A}$ agonists on food intake in rats may be related to the motivational state of the animals to feed.

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649P PRETREATMENT WITH 8-OH-DPAT OR GEPIRONE DOES NOT ATTENUATE THE INHIBITORY EFFECT OF CHOLECYSTOKININ (CCK) ON FOOD INTAKE IN RATS

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Poeschla et al (1992) have recently reported that pretreatment with the $5HT_{1A}$ agonist 8-hydroxy-2 (di-n-propylamino)tetralin (8-OH-DPAT) attenuates the hypophagic action of CCK in rats. As $5HT_{1A}$ agonists decrease 5HT function in the CNS, these workers have suggested that the inhibitory effect of peripherally acting CCK is dependent on intact central 5HT systems. The aim of the present investigation was to see if we could replicate and extend these findings.

Experiment 1. 22h food-deprived male Wistar rats (n=8, b. wt.220 - 320g) were injected with either saline (sal) followed by sal (n=8), sal followed by CCK (6 $\mu g\ kg^{-1}$), 8-OH-DPAT (100 $\mu g\ kg^{-1}$) followed by sal, or 8-OH-DPAT (100 $\mu g\ kg^{-1}$) followed by CCK (6 $\mu g\ kg^{-1}$) (n=8). The 1st injection was given s.c. and the 2nd i.p. A period of 60 min separated the 2 injections. Immediately after the 2nd injection, the rats were placed in the experimental cages where they had free access to food and water, and the amount of food consumed by the animals was measured after 30 min. A repeated measures design was used with each animal receiving all 4 treatments. 2 - 3 days separated successive trials. The data was analysed by ANOVA with repeated measures, and *post-hoc* tests were carried out using the Tukey test.

Experiment 2. A similar experimental protocol as that described in Experiment 1 was used except that the animals (male Wistar rats, n=8, b. wt. 250 - 330g) were pretreated with gepirone (2 mg kg^{-1}) instead of 8-OH-DPAT, and food intake for each animal was measured over a 60 min period. Analysis of the data obtained in Experiment 1 showed significant effects of drug treatment on food intake ($F_{(3,21)}$= 16.390, P<0.001). *Post-hoc* tests revealed that while CCK (6 $\mu g\ kg^{-1}$) caused a significant reduction in food intake (P<0.01) from a control value of 5.3 ± 0.2g to 2.2 ± 0.3g, pretreatment with 8-OH-DPAT (100 $\mu g\ kg^{-1}$) did not attenuate the inhibitory effect of CCK (6 $\mu g\ kg^{-1}$) (Food intake after 8-OH-DPAT + CCK = 2.7 ± 0.6g). 8-OH-DPAT (100 $\mu g\ kg^{-1}$) had no significant effects on feeding when administered alone (Food intake = 4.2 ± 0.3g). Similarly, statistical analysis of the data obtained in Experiment 2 revealed that while CCK (6 $\mu g\ kg^{-1}$) significantly suppressed 60 min food intake (P<0.01) from a control value of 8.8 ± 0.7g to 4.6 ± 0.8g, gepirone (2 mg kg^{-1}) pretreatment had no effect on CCK-induced hypophagia (Food intake after gepirone + CCK = 3.9 ± 0.3g). Gepirone (2 mg kg^{-1}) did not significantly affect food consumption when administered alone (Food intake = 7.1 ± 2.0g).

The results of this study show that in contrast to the results obtained by Poeschla et al (1992), pretreatment with the $5HT_{1A}$ agonists 8-OH-DPAT or gepirone does not attenuate the suppressant effect of CCK on food intake in rats. We have also obtained similar results when we examined the effects of these drugs on the hypophagia induced by CCK in 3h-fasted and non-fasted rats (Ebenezer and Brooman, unpublished results). The present results thus suggest that the suppressant effect of systemically administered CCK on food intake is not dependent on intact central 5HT systems, thus making it unlikely that peripherally acting CCK recruits central 5HT processes to inhibit feeding.

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650P THE 5-HT TERMINAL AUTORECEPTOR IS A 5-HT_{1D} RECEPTOR SUBTYPE IN THE GUINEA-PIG CORTEX

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5-HT terminal autoreceptors have been identified in a variety of species and it is generally acknowledged that they belong to the 5-HT_1 receptor class (Middlemiss *et al*, 1988). The rat autoreceptor is of the 5-HT_{1B} subtype (Engel *et al*, 1986), but differs in pharmacological specificity from that in other species such as guinea-pig and human. Middlemiss and colleagues (1988) suggested that the terminal autoreceptor in the latter species correlated with 5-HT_{1D} receptor binding, from studies with non-selective ligands. Using the potent and selective 5-HT_{1D} receptor antagonist, GR127935 (Skingle *et al*, 1993), we now confirm that the guinea-pig 5-HT terminal autoreceptor is of the 5-HT_{1D} subtype.

Guinea-pig cortical slices were preloaded with 100nM [^{3}H]5-HT. Release was electrically stimulated for two periods, S1 and S2, both consisting of a 2ms biphasic square wave pulse, 20mA in amplitude, at a frequency of 1Hz for 120 pulses. Superfusate samples, in the presence of 1μM paroxetine, were collected every 4 min. Receptor agonists and antagonists were superfused during the S2 period. The S2/S1 ratio of fractional release (FR) was calculated and results expressed as a % of control.

Basal release of [^{3}H]5-HT over an 80 min period was 1.81±0.04 %FR (n=40). Electrical stimulation increased release to 4.54±0.26 %FR for S1 and 4.53±0.20 %FR for S2, with a S2/S1 ratio of 1.09±0.03 (n=16). 5-HT inhibited the stimulated release in a concentration dependent manner, to a maximum of 77±4% at 100nM, with a pEC_{50} of 7.85±0.11 (n=14). Concentrations above 100nM increased basal levels through homoexchange. GR127935 (10nM-1μM) altered basal release, 10nM producing a small but significant decrease (14±2%, n=6, $p<0.05$) and 1μM producing a significant increase (29±1%, n=6, $p<0.001$). In the presence of GR127935 (10nM and 30nM) the 5-HT inhibition of release was attenuated, with apparent pA_2 values of 8.60±0.21 and 8.44±0.08 respectively (n=4).

The effect of GR127935 on basal release indicates that it possess another action, the mechanism of which is unknown. However, although significant this effect is not great in magnitude; the overriding effect of the compound was attenuation of both endogenous and exogenous 5-HT induced inhibition of release. The apparent pA_2 values for GR127935 were very close to the 5-HT_{1D} pK_i value of 8.5 in guinea-pig tissue (Skingle *et al* ,1993) and these observations support the theory that the guinea-pig terminal autoreceptor is of the 5-HT_{1D} subtype.

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651P APPARENT UPREGULATION OF THE HUMAN CLONED 5-HT_{2A} RECEPTOR IN HEK 293 CELLS BY MIANSERIN

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Chronic antagonist treatment paradoxically down-regulates the 5-HT2A receptor (see Rinaldi-Carmona et al, 1992). We have investigated the effects of mianserin (mians) on 3H-ketanserin (Ket) binding to the human cloned 5-HT2A receptor stably expressed in HEK 293 cells (Wood et al, 1993), a model free from transynaptic mechanisms.

HEK 293 cells were grown in minimum essential medium with Earles salts supplemented with 10% foetal calf serum. Mians was added to the medium for varying times prior to harvesting. Cells were harvested by trypsinisation and centrifugation and were washed twice. Membranes were prepared by homogenisation and washed twice with an intermediate incubation at 37oC for 15 min. 3H-Ket binding was performed using standard methods (Wood et al, 1993) and data analysed using LIGAND.

Mians (100nM) increased the Bmax for 3H-Ket, which was significant at 24 h (Table 1), whether the mians was added before or when the cells were confluent(although there a difference in the Bmax for the two conditions (Table 1)). This effect was not attenuated by 5-HT (10μM). A reduction in 3H-Ket affinity was seen with mians treatment in the confluent group (Table 1).

Table 1: The effect of mianserin treatment on 3H-ketanserin binding to human 5-HT2A receptors in a clonal HEK 293 cell line.

Treatment	cells confluent (mean±SEM, n=3)		Pre-confluent cells (mean±SEM, n=5)	
	KD(nM)	Bmax(pmoles/mg protein)	KD(nM)	Bmax(pmoles/mg protein)
Control	0.24 ± 0.04	3.16 ± 0.22	0.26 ± 0.02	1.19 ± 0.27
Mianserin 1 h	0.50 ± 0.09	5.72 ± 0.95	0.28 ± 0.03	1.90 ± 0.38
Mianserin 24 h	0.34 ± 0.01*	5.84 ± 0.20*	0.24 ± 0.03	2.33 ± 0.48*
Mianserin+5-HT 24 h	0.36 ± 0.02*	5.95 ± 0.80*	0.35 ± 0.12	1.93 ± 0.52

* $p<0.05$ compared to control, Student's paired t-test

Mians blockade of the 5-HT2A receptor in a clonal cell line results in an apparent up-regulation. This agrees with findings with mians in human platelets (Whiteford et al, 1993) and with the 5-HT2A selective antagonist SR 46349B in mouse brain (Rinaldi-Carmona et al, 1992). The down-regulation seen in-vivo may therefore be due to effects of mians other than 5-HT2A receptor blockade.

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652P FORE-PAW TREMOR AND FORE-PAW LICKING INDUCED BY THE TRH ANALOGUE MK771: MODULATION BY 5-HT$_2$ RECEPTOR ANTAGONISTS

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We have previously demonstrated that the TRH analogue MK771 induces fore-paw-licking (FPL) in mice, which is antagonised by low doses of 5-HT$_{1A}$ agonists (McCreary and Handley, 1993). The induction of fore-paw-tremor (FPT) in rats has also been noted (Simasko and Horita, 1984). In rats FPL induced by administration of CG3509 was attenuated by prior administration of the 5HT$_2$ receptor antagonist ritanserin (Fone *et al.*, 1989). Therefore, we have investigated the effects of the TRH analogue MK771 (Veber *et al.*, 1976) on FPT and further analysed the effects of 5-HT$_2$ antagonists on FPT and FPL.

Male MF1 mice (20-30g>8/group) were pre-treated with ritanserin (Rit) (1.0 and 5.0mg/kg i.p.), ketanserin tartrate (Ket) (1.0 and 5.0 mg/kg s.c.), mianserin (Mian) (2.0 mg/kg s.c.) or saline 7.5 min. prior to the administration of MK771 (2.5 mg/kg i.p.) (MK) and the number of FPT and FPL bouts counted 7.5 min. later for a 5 min. time epoch. Behaviour was recorded on video tape and analysed later with the experimenter unaware of the treatment that each mouse had received.

Table 1: Effects of ritanserin (rit), ketanserin (ket) and mianserin (mian) on FPT and FPL induced by MK771 (mean bouts ± s.e.m.).

Drug/ dose(mg/Kg)	Sal	Rit 1.0	Sal	Rit 5.0	Sal	Ket 1.0	Sal	Ket 5.0	Sal	Mian 2.0
FPT (bouts)	35.63± 6.34	20.50± 5.67§	48.75± 3.69	31.42± 4.70*	47.42± 9.92	23.76± 8.05NS	45.26± 6.90	21.79± 2.22*	47.42± 9.92	17.18± 3.74q
FPL (bouts)	10.72± 1.72	2.83± 0.87**	12.30± 3.75	4.27± 1.60§	10.86± 3.04	5.51± 2.51NS	12.46± 2.19	0.28± 0.20**	10.85± 3.04	2.43± 0.96@

**, p≤0.0005; *, p≤0.005; @, p≤0.025;q, p≤0.01; §, p≤0.05 and NS, not significantly different from saline control group (unpaired t-test).

As shown in table 1, all three agents significantly reduced both FPT and FPL, suggesting the involvement of 5-HT$_2$ receptors in modulating these behavioural effects of MK771.

Acknowledgements. We thank the Tourette's Syndrome Association (U.S.A.) for financial support.

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653P ATYPICAL NEUROLEPTIC AGENTS ATTENUATE THE INHIBITORY PROFILE OF 5-HYDROXYTRYPTOPHAN IN THE MOUSE LIGHT/DARK TEST

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Many neuroleptic agents have nanomolar affinity for the 5-HT$_{2A/2C}$ receptors (Leysen, 1992). The present study investigates the ability of atypical and typical neuroleptic agents to modify the ritanserin sensitive inhibitory action of 5-hydroxytryptophan in the light/ dark test (Costall *et al.*, 1993).

Male albino BKW mice (Bradford strain) received an intraperitoneal injection of vehicle, 5-hydroxytryptophan (5-HTP, 50mg kg^{-1}), ritanserin (1.0mg kg^{-1}), clozapine (1.0mg kg^{-1}), thioridazine (1.0mg kg^{-1}) and benperidol (0.16mg kg^{-1}) according to the regimens shown in Table 1. Drugs were administered as 40 min pretreatments with testing 40 min after the last treatment. Mice were placed into the light compartment of the light/dark test box and the % time spent and line crossings in the two chambers and the latency of first movement from the light (L) to the dark (D) compartment was recorded by video camera over a 5 min period (for methodological details see Costall *et al.*, 1989) and are shown in Table 1.

Table 1. The action of 5-HTP and its interaction with ritanserin and neuroleptic agents to inhibit and disinhibit mouse behaviour in the light/dark test box

Treatment	Latency L>D (s)	% time in L	Line crossings/5 min L	Line crossings/5 min D
Vehicle	11.2 ± 1.6	46 ± 5.8	24 ± 3.3	65 ± 8.2
5-HTP	2.2 ± 0.8*	11 ± 2.3*	8 ± 3.6*	125 ± 15.0*
Ritanserin + 5-HTP	9.9 ± 1.4†	51 ± 6.2†	31 ± 4.8†	54 ± 7.3†
Clozapine + 5-HTP	24.7 ± 3.0*†	76 ± 9.5†	36 ± 5.1†	73 ± 8.5†
Thioridazine + 5-HTP	18.4 ± 1.7*†	69 ± 5.6†	38 ± 5.0†	58 ± 7.1†
Benperidol + 5-HTP	3.4 ± 1.5*°	12 ± 4.0 °*	12 ± 2.4°*	7.5 ± 5.5°*

n = 10. A significant decrease or increase compared to the vehicle response is shown *P<0.05-0.001; a significant antagonism compared to 5-HTP is shown †P<0.001 (one way ANOVA followed by Dunnett's t test). °Non-specific locomotor depression.

The 5-HTP induced decrease in behaviour in the light compartment of the test box was antagonised by ritanserin, clozapine and thioridazine, the inhibition being reversed to disinhibition. When administered alone the latter agents failed to modify mouse behaviour in the test box (data not shown, n = 10, P>0.05). Non-specific locomotor depressant effects of benperidol obscured an interpretation of its effects to modify the inhibitory effects of 5-HTP. All the neuroleptic agents used in this study have affinity for both dopamine and 5-HT$_{2A/2C}$ receptors: the latter action may contribute to the actions of clozapine and thioridazine in the presence of 5-HTP to disinhibit mouse behaviour in the test box.

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654P [^{3}H]GR113808 BINDING TO 5-HT$_4$ RECEPTORS IN HUMAN NEURODEGENERATIVE DISORDERS

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The regional distribution and pharmacology of [^{3}H]GR113808 binding to the 5-HT4 receptor in human brain tissue has recently been reported (Waeber et al., 1993; Reynolds et al., 1994). The high receptor density in basal ganglia as well as the moderate levels observed in limbic (including hippocampal) and neocortical regions, indicates the potential involvement of 5-HT$_4$ receptors in motor and cognitive functions (Waeber et al., 1993). We have employed [^{3}H]GR113808 binding to determine the density of 5-HT$_4$ receptors in brain tissue obtained post-mortem from patients with motor and cognitive neurodegenerative disorders. Tissue from Huntington's (HD), Parkinson's (PD) and Alzheimer's (AD) patients were studied, each series with an appropriately age-matched control group. Tissue preparation and [^{3}H]GR113808 binding were performed as previously reported (Reynolds et al., 1994). Non-specific binding was defined in the presence of 100 µM 5-HT. Receptor binding was undertaken with two ligand concentrations, 0.05 and 0.2 nM. As the mean K_d values for the tissues studied were in the range of 53-73 pM, the higher concentration relates to approximately 73-80% of receptor occupancy, while the ratio of binding at the two concentrations was determined by receptor affinity. No significant difference in the binding ratios between the control and disease groups was observed, indicating that differences in binding may be ascribed to differences in 5-HT$_4$ receptor density.

Table 1 [^{3}H]GR113808 binding to post-mortem brain tissue in neurodegenerative disorders

	Parkinson's disease		Huntington's disease	Alzheimer's disease	
Brain region:	Putamen	Sub. nigra	Putamen	Hippocampus	Frontal cortex
Patients	5.86±1.48 (6)	5.57±0.10 (6)	2.68±1.08 (6)**	0.78±0.61 (8)**	1.30±0.22 (8)*
Controls	4.74±0.87 (6)	4.21±2.56 (6)	5.33±1.00 (6)	2.34±0.62 (8)	1.76±0.19 (6)

Values are mean±S.D. (number of separate experiments) of specific binding at 0.2 nM [^{3}H]GR113808 (fmole/mg tissue). **$p < 0.001$, *$p < 0.01$ by Student's t-test.

The absence of any deficit in binding in PD brain tissue (Table 1) indicates that the 5-HT$_4$ receptor is not primarily located on dopaminergic cell bodies or terminals that are lost in this disorder. However, a 50% deficit in binding to the putamen in HD, and decreases in binding of 67% (hippocampus) and 26% (frontal cortex) in AD were observed. Since GABAergic deficits are the most prominent feature of HD, the results might be due to GABAergic neuronal loss, although there are reductions in both glutamate and the cholinergic marker, choline acetyltransferase, in HD striatum (Reynolds et al., 1990). The findings in AD brain tissue closely reflect the neuropathology of the disease, in which hippocampal atrophy is most profound. The results may reflect loss of cholinergic terminals, but could also relate to loss of glutamatergic (Proctor et al., 1988) or GABAergic (Rossor et al., 1986) neurones.

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655P THE 5-HYDROXYTRYPTAMINERGIC SYSTEM IN ALZHEIMER'S DISEASE EXHIBITS PLASTICITY AND IS AFFECTED BY NEUROLEPTIC MEDICATION

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In 20 prospectively assessed, pathologically confirmed, Alzheimer's Disease (AD) cases and 17 age and postmortem delay matched controls (CON), 5-HT neurones stained with an antibody to tryptophan hydroxylase (Halliday *et al*, 1992) were counted in the dorsal raphe nucleus (DRN), and structural and functional markers of the 5-HT innervation were measured by [^{3}H] paroxetine (PRX) binding (Backström *et al*, 1989) and HPLC respectively in the orbito-frontal (BA11) and temporal (BA21) cortex.

Despite a loss of 5-HT neurones in the DRN (CON 653±42; AD 371±27; p<0.001), PRX B_{max} (fmol/mg ± s.e. mean) in BA11 was unchanged (CON 88.9±10.7; AD 76.8±10) but reduced in BA21 (CON 64.1±6; AD 40.9±3.6; p=0.002). PRX K_d was unaltered. As the DRN has highly collateralised projections to the primate association cortex (Wilson & Molliver, 1991), sprouting of the 5-HT innervation may occur in regions less affected by AD pathology. The concentration of 5-HT (pmol/mg ± s.e. mean) in AD was unaltered (BA11: CON 1.6±0.2; AD 1.5±0.2; BA21: CON 0.9±0.1; AD 0.7±0.1) but the 5-HIAA/5-HT ratio was significantly higher (BA11: CON 11.6±1.4; AD 17.9±2.4; p=0.03; BA21: CON 13.8±1.4; AD 22.8±3.2; p=0.04) suggesting an increase of turnover in the surviving 5-HT innervation. The correlation between PRX B_{max} and 5-HT in controls (n=17; r=0.45; p=0.03) was lost in neuroleptic (NL) free AD cases (n=12; r=0.26; p=0.21) but retained in those on chronic neuroleptic treatment (n=8; r=0.6; p=0.014) who also had a lower 5-HT (BA11: NL 1.1±0.2; NL-free 1.8±0.3; p=0.04; BA21: NL 0.5±0.1; NL-free 0.9±0.1; p=0.1) and a reduced sum of 5-HT + 5-HIAA (BA11: NL 20.3±3.8; NL-free 27.3±3.2; p=0.18; BA21: NL 10.9±1.6; NL-free 17.8±2.2; p=0.04). Thus, neuroleptic treatment may affect 5-HT turnover in AD.

If 5-HT has a role in the cognitive and behavioural features of AD, then these findings may have potential therapeutic implications.

Supported by the MRC and the Oxford District Health Authority Research Committee.

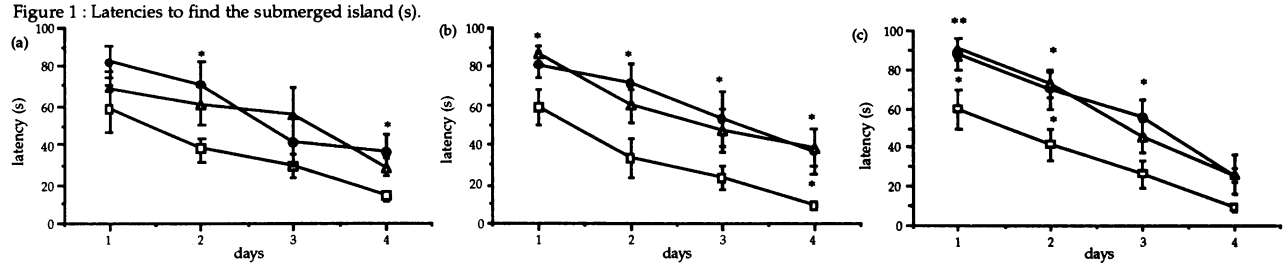
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656P FAILURE OF PHYSOSTIGMINE TO AFFECT THE WATER MAZE ACQUISITION DEFICIT PRODUCED BY COMBINED pCPA AND SCOPOLAMINE TREATMENT

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The combined administration of parachlorophenylalanine (pCPA) and scopolamine disrupts the acquisition of a water maze task in rats. The behavioural deficit was not reversed by treatment with 5-HTP (Harder & Kelly, 1993). Current studies examined the effect of the anticholinesterase, physostigmine, alone and in combination with 5-HTP, on the impairment produced by the combined treatment. Male Lister Hooded rats were trained for 4 trials per day over 4 days in a water maze following 3 days of pCPA treatment (100mg/kg ip daily). Physostigmine (0.3mg/kg or 0.5mg/kg ip) was administered 15 min before maze exposure, 5-HTP (50mg/kg, ip) as a 30 min pretreatment and scopolamine (scop; 0.25mg/kg, ip) as a 20 min pretreatment (doses chosen as ineffective to modify behaviour alone). The time taken to locate the submerged island (latency; s) was used as the measure of learning ability.

Figure 1 : Latencies to find the submerged island (s).



Data are presented as mean ± s.e.mean, n=7-9. -□- vehicle, -●-pCPA+scop, -△- pCPA+scop+physostigmine (+5-HTP in figure 1(c)); (a) 0.3mg/kg physostigmine, (b) 0.5mg/kg physostigmine, (c) 0.3mg/kg physostigmine + 50mg/kg 5-HTP. *p<0.05, **p<0.01 compared to vehicle control group; one factor ANOVA followed by Dunnett's t test.

In all 3 experiments, combined pCPA and scopolamine treatment produced a significant impairment in the acquisition of the water maze task, as shown by the increased latency relative to vehicle controls (see Figure 1a-c). Neither physostigmine alone nor 5-HTP and physostigmine in combination could alleviate the impairment. No significant differences were observed between pCPA/scopolamine treatment and pCPA/scopolamine/physostigmine treatment in either experiment; similarly, there were no significant differences between pCPA/scopolamine and pCPA/scopolamine/5-HTP/physostigmine treatment.

A previous study has shown that treatment with 5-HTP could not reduce the water maze deficit produced by combined pCPA and scopolamine treatment (Harder & Kelly 1993). Current studies suggest that physostigmine treatment alone is also ineffective and in addition a combined 5-HTP and physostigmine treatment is insufficient to reverse the cognitive deficit.

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657P RETARDED ACQUISITION OF A TEMPORAL DISCRIMINATION FOLLOWING TREATMENT WITH DSP4

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The ascending noradrenergic and 5-hydroxytryptaminergic (5HTergic) pathways are believed to play opposing roles in a number of behavioural functions (Ogren, 1985; Wogar *et al.*, 1991). Recently we reported that destruction of the 5HTergic pathways resulted in accelerated acquisition of a temporal discrimination (Graham *et al.*, 1994). Here we report the effect of the selective noradrenaline neurotoxin DSP4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine) on the acquisition of a temporal discrimination using an interval bisection task (Morrissey *et al.*, 1993).

18 rats received 2 injections of DSP4 (50 mg kg^{-1} i.p.) separated by 7 days; 18 rats received vehicle injections (0.9% NaCl). The rats were then trained to press levers in operant conditioning chambers using a sucrose reinforcer (50 µl, 0.6 M). In Phase I (25 days), each daily 35-minute session consisted of 100 discrete trials in which the rats were required to press lever A following a 2-s presentation of a light stimulus, and lever B following an 8-s presentation of the same stimulus. In Phase II (25 days), 10 'probe' trials (without reinforcement) were introduced into each session, in which the light was presented for intermediate durations (2.5, 3.2, 4.0, 5.0, 6.4 s; 2 trials in each case). After completion of the experiment, the rats were sacrificed and their brains dissected for determination of the concentrations of 5HT, 5-hydroxyindoleacetic acid (5HIAA), noradrenaline and dopamine by high-performance liquid chromatography with electrochemical detection (for method, see Morrissey *et al.*, 1993).

During Phase I both groups showed gradual acquisition of accurate discrimination, attaining 90% correct choices within 15 sessions. Analysis of variance revealed that the DSP4-treated group learned the discrimination significantly more slowly than the control group (group: $F[1,34]=8.9$, $P<0.01$; sessions: $F[24,816]=150.9$; $P<0.001$; interaction: $F[24,816]=1.6$, $P<0.05$), the between-group difference being statistically significant ($P<0.05$) in sessions 4-9. During Phase II the bisection point (duration corresponding to 50% choice of lever B, ± s.e.mean) did not differ significantly between the DSP4-treated (3.9 ± 0.1 s) and control (4.0 ± 0.1 s) groups ($P>0.1$). The Weber fraction, an index of discriminative accuracy (Morrissey *et al.*, 1993), was significantly lower in the DSP4-treated group (0.202 ± 0.016) than in the control group (0.255 ± 0.012) ($t[34]=2.6$, $P<0.05$). The levels of noradrenaline in the parietal cortex and hippocampus were reduced to about 20% of control values in the DSP4-treated group (ng g^{-1} wet weight of tissue, mean ± s.e. mean: *parietal cortex*, 51 ± 8 [DSP4-treated], 261 ± 20 [control], $t[34]=9.8$, $P<0.001$; *hippocampus*, 47 ± 7 [DSP4-treated], 324 ± 24 [control], $t[34]=11.0$, $P<0.001$). The levels of dopamine, 5HT and 5HIAA did not differ significantly between the groups.

The results show that treatment with DSP4 retarded the learning of temporal discrimination, but did not impair steady-state discriminative accuracy.

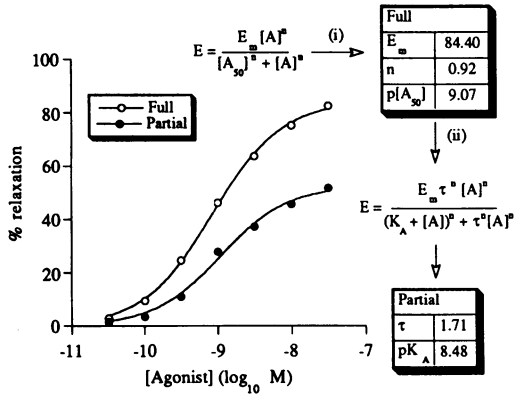
This work was supported by the Medical Research Council.

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658P DEMONSTRATION OF A PERSONAL COMPUTER BASED PROGRAM FOR DIRECT OPERATIONAL MODEL-FITTING

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The operational model of agonism (Black & Leff, 1983) provides a means of directly analysing raw experimental concentration-effect (E/[A]) curve data for the purpose of estimating agonist affinities (pK_A) and efficacies (τ). Typically, this type of analysis has been carried out by simultaneously fitting two or more E/[A] curves to the model equations describing the comparative, receptor inactivation or interaction methods (Leff *et al*, 1990; Leff *et al*, 1993). Such procedures have generally necessitated the use of powerful statistical systems and a mainframe computer.
The purpose of this demonstration is to illustrate that reliable affinity and efficacy estimates are possible, without a mainframe or simultaneous fitting procedures. Running on a personal computer, this method makes model-fitting available to a wider audience.



The application we have used is the data analysis/graphics package, KaleidaGraph (version 3.0), for the Macintosh series of personal computers (the principle used should however, apply to any suitable package that has user defined functions and iterative capabilities). Initially, experimental E/[A] curve data is imported from the relevant Microsoft Excel (version 4.0) spreadsheet and displayed in a graphical format. The data for the standard full agonist is then fitted to a logistic using the 'General Curve Fit' option to obtain estimates for the asymptote (E_m), slope (n) and location ($p[A_{50}]$). The required parameters are then substituted into the appropriate model equation for subsequent analysis of the test curve data. The figure shows the procedure illustrated for one of the three possible cases, the comparative analysis.

Fig 1. Analysis of a partial agonist using the sequential operational model-fitting procedure. Data for the full agonist is fitted to a logistic (i) to obtain E_m and n estimates. E_m and n are then set to these values in the operational model equation (ii) to allow K_A and τ to be estimated. Data shown - PGE_2 (○) and 11-deoxy-16,16-dimethyl PGE_2 (●) in the rabbit isolated saphenous vein.

Black, J.W. & Leff, P. (1983). *Proc. R. Soc. Lond. B.*, **220**, 141-162.
Leff, P., Prentice, D.J., Giles, H., Martin, G.R. & Wood, J. (1990). *J. Pharmacol. Methods*, **23**, 225-237.
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659P OPTIMA: SIMULTANEOUS FITTING OF RESPONSE CURVES TO THE OPERATIONAL MODEL OF AGONISM WITH MICROSOFT EXCEL

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The operational model is a theoretical model for agonist action, that can be fitted directly to agonist concentration-effect data to allow estimation of affinity and efficacy. To obtain reliable estimates of each parameter, it is necessary to apply it simultaneously to multiple receptor agonist response curves, allowing for the sharing of some parameters between each fitted curve. We have developed an iterative analytical procedure within Microsoft Excel, called OPTIMA, which can be run on IBM compatible PC's or on the Macintosh.

The equation for the operational model (Table 1) contains the parameters [A] = agonist concentration, Em = maximum possible effect in the system, KA = equilibrium dissociation constant, τ = operational quantity defining efficacy, and n = slope factor (Black & Leff, 1983). Values for effect (E) are predicted from this equation using initial estimates of Em, KA, n and τ. The difference between the observed and predicted value of E is squared, summed for the data within the curve, and divided by the number of points to give the mean square difference (MSD)(Table 1). Where several curves are being analysed simultaneously the MSD for each curve is calculated, then an overall MSD is obtained as a mean of these. The data can then be fitted iteratively using the Solver function within Excel. This is programmed to minimise the overall MSD by changing the values of Em, KA, n (these remain the same for all curves), and also that of τ which is allowed vary for each curve. When changes to the variables do not result in a significant change in the overall MSD, Solver reports the final values for each variable. OPTIMA reports the fitted data, and produces a graphical representation of each predicted curve.

This method of analysis allows use of the operational model of agonism with minimal computing costs and programming. It can be extended to other models which require that binding/response isotherms be fitted simultaneously. Statistical analysis of fitted data is a key objective for future development of OPTIMA.

Black & Leff (1983) Proc. R. Soc. Lond. B., 220, 141-162.

Table 1. Example of data fitted to the operational model of agonism shown below.

$$E = \frac{Em\tau^n[A]^n}{(K_A+[A])^n+\tau^n[A]^n}$$

Log [A]	Observed	Predicted	Sqr Diff
-11	0.0	0.43	0.18
-10.52	1.2	1.59	0.15
-10	6.4	6.37	0.00
-9.52	22.6	20.38	4.95
-9	47.4	51.58	17.51
-8.52	82.5	79.42	9.46
-8	96.2	93.34	8.16
-7.52	99.3	97.30	4.00
		MSD	5.55

τ = 140.43 Em = 99.2
KA = -6.88 n = 1.2

660P TEACHING FUNCTIONAL ANATOMY OF THE RAT USING AN INTERACTIVE LASER VIDEO DISK

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An interactive CAL program developed using HyperCard™ has already been described (Quentin-Baxter & Dewhurst, 1992). This program was developed to run on the Macintosh® Plus microcomputer with two, floppy disk drives and HyperCard™ and its development was therefore constrained by a monochrome monitor and the need to run from a single floppy disk (800kb). Subsequent evaluation of this software highlighted the need to use high quality colour images in order to maximise the learning experience. Here we describe a low cost, two-screen interactive laser video of an extensive rat dissection which combines interactivity and full colour images and which is designed to enhance the teaching of functional anatomy for students of biology at high school/undergraduate level.

The dissection video is a high quality, 34 minute laser video disk illustrating aspects of the dissection of both male and female rat and is played from one side of a 12" PAL laser disc which offers rapid information retrieval and accurate playback. Short sequences of moving, full colour video are punctuated with high quality still frames to demonstrate dissection techniques and to illustrate important mammalian anatomical features. The main dissection is depicted from above and this view supports three levels of picture 'zoom', (the whole animal in the picture, half the animal filling the picture, and the picture encompassing a single organ), to allow detailed examination of the dissection while maintaining a sense of scale. A secondary view of the dissection, from the side and at the level of the animal, offers a three dimensional quality to the presentation. It covers external anatomy, the abdomen (gastrointestinal, urinary and reproductive structures) the thorax and neck and the brain. In addition the disk contains an extensive range (300 individual images) of mammalian histology sections showing the cellular detail (in up to four magnifications) of various mammalian tissues.

The video disk may be used in a highly interactive way via a HyperCard™ stack (which contains about 250 black and white images) running on any 4/40 MB Macintosh® (SE or better). Text and high quality images (digitised from key images on the resource disk) are presented on the computer screen and are supported by a large amount of on-line, Hypertext information which is accessed in an highly interactive way. The software allows full control over the laser disc and offers facilities such as three levels of magnification of certain images, views of the dissection from both above and the side, and the opportunity to interchange views from animals of either sex.

The stack, which may be modified or tailored by the teacher, is fully functional without the video disk present and may be used independently of the video disc. In addition, the visual images contained on The Rat Anatomy Disk may be seen as a stand alone resource around which new interactive software may be developed. It is anticipated that it will be used in a student centred way, with students directing the content and sequence of material presented to them. The material described may supplement actual animal dissection and, in some instances, provide a realistic alternative means of achieving many of those learning objectives traditionally addressed by performing a mammalian dissection.

Quentin-Baxter, M. & Dewhurst, D.G. (1992) J. Biol. Educ. **26 (1)**, 27-33.

661P CONFOCAL LOCALISATION OF BODIPY FL-PRAZOSIN BINDING IN RAT MESENTERIC AND CEREBRAL RESISTANCE ARTERIES

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ORAL COMMUNICATIONS

In oral communications with more than one author, the first author is the one who intended to present the work

1P **Belelli D, Callachan H, Lambert JJ, Peters JA, Hill-Venning C, Lan NC & Gee KW** A comparative study of the influence of allosteric modulators upon human ($\alpha_3 \beta_1 \gamma_{2L}$) and *Drosophila* recombinant GABA receptors

2P **Luque JM, Erat R, Kettler R, Cesura AM, Malherbe P, Da Prada M & Richards JG** $GABA_A$ receptor subunit mRNAs and [^{3}H]SR 95531 binding sites in the locus coeruleus

3P **Sanger DJ, Joly D, Perrault G & Morel E** Do benzodiazepine (BZ) (w) receptor partial agonists disrupt the acquisition of learned fear in mice?

4P **Watson WP & Little HJ** Identification of distinct components, with different time courses, of convulsion threshold changes during ethanol withdrawal

5P **Smith SE, Parvez NS, Al-Zubaidy ZA & Meldrum BS** Anti-convulsant effects of tiagabine and NNC-711 in the genetically epilepsy-prone rat

6P **Dhillon A & Jones RSG** Modification of synaptic responses of entorhinal cortical neurones by a new $GABA_B$ antagonist, CGP 55845

7P **Bernasconi R, Mathivet P, Marescaux C, Leonhardt T, Martin P, Mickel S & Froestl W** NMDA receptors and NO synthase (NOS) are involved in the increase of cerebral cGMP induced by $GABA_B$ antagonists

8P **Thurlow RJ, Hill DR & Woodruff GN** Further characterisation of gabapentin binding sites in brain

9P **Bristow LJ, Coomber A, Hogg J & Hutson PH** The glycine/NMDA receptor antagonists, R-(+)-HA966 and 5,7 dichlorokynurenic acid attenuate naltrexone-precipitated morphine withdrawal in the rat

10P **Dijk S, Francis PT & Bowen DM** NMDA-induced glutamate release from rat cortical pyramidal neurones is potentiated by a 5-HT_{1A} antagonist

11P **Teoh H, Malcangio M, Fowler LJ & Bowery NG** Effect of lamotrigine on glutamate and substance P release from the rat spinal cord

12P **Tarnawa I & Farkas S** *In vivo* and *in vitro* electrophysiological actions of novel 2,3-benzodiazepine AMPA antagonists

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14P **Chapman AG, Smith SE, Parvez N & Meldrum BS** The effect of aniracetam and cyclothiazide on the anticonvulsant action of four AMPA antagonists in rodent seizure models

15P **Stanhope KJ, Choules M, Yudko E & Dourish CT** Re-evaluation of the effects of putative cognitive disrupters in the reinforced-alteration T-maze task in the rat

16P **Panegyres PK, Poat J & Hughes J** A comparison of the effects of enadoline and dizocilpine on immediate early gene expression in the central nervous system

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18P **Sanz MJ, Weg VB & Nourshargh S** Mechanisms of eosinophil accumulation induced by interleukin-1 in rat skin: evidence for the involvement of PAF

19P **Moore HD, Wassermann L, Kern EM & Bevan D** Inflammatory mediators of irritant contact dermatitis from mouse ears

20P **Harris JG, Perretti M & Flower RJ** Histamine co-operates with interleukin-8 to induce neutrophil accumulation into the mouse air-pouch

21P **Au B-T, Williams TJ & Collins PD,** Role of the CD11b/CD18 receptor and platelet activating factor in the regulation of interleukin-8 release from zymosan-stimulated human neutrophils

22P **Teixeira MM, Norman KE, Fairbairn SM, Williams TJ, Rossi AG & Hellewell PG** Mechanisms of immune complex-induced inflammation in guinea-pig skin

23P **Rossi AG, Williams TJ, Kirk SA, McDonald F & Pons F** Pro- and anti-inflammatory effects of the stable PGD analogue, ZK 118.182

24P **Smith SF, Datta AK, Smith T, Tetley TD, Guz A & Flower RJ** Lipocortin 1 levels in lung lavage fluids collected from healthy volunteers before and after taking exogenous glucocorticoids

25P **Talpain E, Armstrong RA, Vardey C & Coleman RA** Characterisation of the PGE receptor subtype mediating inhibition of superoxide production in human neutrophils

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31P **Graham A, Saville MK, Malarkey K, Gould GW & Plevin R** Lysophosphatidic acid stimulates the tyrosine phosphorylation of focal adhesion kinase $pp125^{FAK}$ in Rat-1 fibroblasts

32P **Baxter GF & Yellon DM** Protection against ischaemic injury in rabbit myocardium 24 hours after adenosine A_1 receptor activation

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34P **Lightbown ID, Cooper S, Lad N & MacKenzie I** Cardiac effect of glibenclamide *in vitro* and *in vivo*: a comparison with compounds related to AZ-DF 265

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36P **Laycock SK, Kane KA, *McMurray J* & Parratt JR** The differential effect of captopril on the cardiac hypertrophic and inotrophic effects of chronic noradrenaline treatment in rats

37P **Rakovic S & Terrar DA** Influence of isoprenaline on the sarcoplasmic reticulum contribution to calcium transients in myocytes isolated from guinea-pig ventricle

38P **Brodde O-E, Zerkowski H-R, Schranz D, Broede-Sitz A, Michel-Reher M & Schäfer-Beisenbusch** Age-dependent decrease of β-adrenoceptor function in human right atrium

39P **Summers RJ, Matthews JM & Molenaar P** Examination of transregulation of β-adrenoceptors and muscarinic cholinoceptors in rat heart

40P **Pfaffendorf M, Batink HD, Kam KL, Pijl AJ, Mathy M-J & van Zwieten PA** Characteristics of β-adrenoceptors in cardiac tissue from spontaneously hypertensive rats with streptozotocin-induced diabetes mellitus

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43P **Grocott-Mason RM, Anning PB, Lewis MJ & Shah AM** Captopril enhances ventricular relaxation via endogenous bradykinins

44P **Anderson IK & Hilditch A** Comparison of the cardiovascular effects of the angiotensin AT_1 receptor antagonists GR138950 and losartan with enalapril in conscious renal hypertensive rats

45P **Richer C, Vacher E, Fornes P, Bruneva P & Giudicelli JF** Early and late effects of angiotensin II subtype 1 receptor blockade on haemodynamics and cardiovascular remodelling in spontaneously hypertensive rats

46P **Hadoke P, Wadsworth RM & Wainwright CL** Alterations in function and morphology of the rabbit subclavian artery following angioplasty

47P **Schoeffter P, Seuwen K, Kaupmann K, Bruns C, Lübbert H & Hoyer D** Four human somatostatin (SRIF) receptors (SSTR-1 to SSTR-4) are negatively coupled to adenylyl cyclase

48P **Ball DI, Beresford IJM, Wren GPA, Pendry YD, Sheldrick RLG, Walsh DM, Turpin MP, Hagan RM & Coleman RA** *In vitro* and *in vivo* pharmacology of the non-peptide antagonist at tachykinin NK_2-receptors, GR159897

49P **Stratton SC, Beresford IJM & Hagan RM** GR159897, a potent non-peptide tachykinin NK_2 receptor antagonist, releases suppressed behaviours in a novel aversive environment

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53P **Haworth D, Thomson DS, Dutta AS & Gormley JJ** Agonist activity and selectivity of [Phe^{36}]-neuropeptide Y(13-16) in anaesthetised, areflexic rats

54P **Wisskirchen FM & Marshall I** Human α-calcitonin gene-related peptide (hα-CGRP) causes an endothelium-dependent vasorelaxation in the rat isolated renal and pulmonary artery

55P **Wenham D & Marshall I** α_{1A}- and α_{1B}-adrenoceptor subtypes and contractions of the rat thoracic aorta to noradrenaline, phenylephrine and oxymetazoline

56P **Kirkup AJ, Edwards G & Weston AH** The effects of niflumic acid on noradrenaline-induced chloride currents and mechanical responses of the rat portal vein

57P **Hendriks MGC, Pfaffendorf M & van Zwieten PA** The effect of 1,4-dihydropyridines on α_1-adrenoceptor agonist- and depolarisation-induced contractions of perfused resistance arteries

58P **Munavvar AS & Johns EJ** The role of α_1-adrenoceptor subtypes in the regulation of renal tubular processes in the 2K1C Goldblatt hypertensive rats

59P **Michel MC & Insel PA** Comparison of drug affinities at cloned and rat tissue α_1-adrenoceptor subtypes

60P **Agnel M, Pimoule, Esnaud H, Langer SZ & Graham D** Pharmacological characterisation and tissue distribution of human α_2-adrenoceptor subtypes

61P **Sithers AJ, Marshall RW & Spriggs TLB** A stimulatory action of chloroethylclonidine in the response of the rat vas deferens to single pulse field stimulation (SPFS)

62P **Allen MJ & Coleman RA** β_2-adrenoceptors mediate inhibition of thrombin-stimulated increases in endothelial cell monolayer permeability

63P **Matthews EK, Seaton E, Forsyth MJ & Humphrey PPA** Effects of a photosensitive nitric oxide donor on isolated guinea-pig taenia coli

64P **Rae MG & Muir TC** Both ATP and nitric oxide (NO) may be involved in the neuronally-mediated relaxation of the guinea-pig internal anal sphincter (gpIAS)

65P **Matthews JS, Keen M & Key BJ** Chronic exposure to the nitrovasodilators glyceryl trinitrate (GTN) or 3-morpho-linosydnonimine (SIN-1) inhibits prostacyclin release from endothelial cells

66P **Drieu la Rochelle C, Grosset A & O'Connor SE** Comparison of the haemodynamic profiles of structurally-unrelated nitrovasodilators in anaesthetised dogs

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68P **Laszlo F, Whittle BJR & Moncada S** Interactions between endogenous nitric oxide, platelet activating factor and thromboxanes during endotoxin-induced acute vascular injury in rat intestine

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70P **van Gelderen EM, De Bruijne ELE, Agteresch H & Saxena PR** Differential effect of nitric oxide donors on the distribution of the common carotid blood flow in the cranial circulation of the pig

71P **Freemantle CN, Buttery LDK, Springall DR, Riveros-Moreno V, Polak JM & Piper PJ** Inhibition of expression of inducible nitric oxide synthase and reduction of blood flow in murine tumours by dexamethasone

72P **Smith JA & Babuji RJ** Oxidised, but not native, low density lipoprotein activates endothelial cell protein kinase C

73P **Elliott J** The effect of streptozotocin-induced diabetes on agonist-stimulated and flow-stimulated release of nitric oxide in the mesenteric circulation of the rat

74P **Stücker O, Pons C, Duverger JP, Hamon G, Wang X & Trouvé R** Impairment of arteriolar sensitivity to iloprost in diabetic rats: partial restoration by a chronic treatment with trandolapril

75P **Carey F, Mirrlees DJ, Sennitt C, Tuffin D, Ward WH, Brittain DR, Howe R & Preston J** Biochemical pharmacology in the rat of the novel aldose reductase inhibitor ZD5522

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77P **Williams TJ, Griffiths-Johnson DA, Collins PD, Walsh DT, Rossi AG, Moqbel R, Truong O, Totty NF, Hsuan J & Jose PJ** A novel eosinophil-specific chemoattractant cytokine generated in an *in vivo* model of allergic asthma in the guinea-pig

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79P **Ward JK, Barnes PJ, Tadjkarimi S, Kelly JJ, Yacoub MH & Belvisi MG** Non-adrenergic non-cholinergic neural relaxation in human tracheal smooth muscle is mediated by an increase in cGMP: further evidence for the role of nitric oxide

80P **Chopra LC, Twort CHC & Ward JPT** Inhibition of contraction by PKC inhibitors, Ro31-8220 and Ro31-75490, in small and large bronchioles of the rat

81P **Hirst SJ, Barnes PJ & Twort CHC** Differential proliferation of rabbit cultured tracheal smooth muscle by platelet-derived growth factor isoforms: inhibition by selective inhibitors of protein kinase C and protein tyrosine kinase

82P **Milne AAY & Piper PJ** The role of the VLA-4 integrin in a model of airway inflammation in the guinea-pig

83P **Lagente V, Moodley I, Perrin S, Mottin G & Junien JL** Effects of isozyme selective phosphodiesterase inhibitors and theophylline on eosinophil infiltration in the guinea-pig lung

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91P **Fletcher A, Bill DJ, Cliffe IA, Forster EA, Jones D & Reilly Y** A pharmacological profile of WAY-100635, a potent and selective 5-HT_{1A} receptor antagonist

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93P **Mundey MK, Fletcher A & Marsden CA** Effect of the putative 5-HT_{1A} antagonist WAY100635 on 5-HT neuronal firing in the guinea-pig dorsal raphé nucleus

94P **Bosch AI, van Hove N, Long SK & de Rond EJ** Systemic flesinoxan depresses hippocampal 5-HT release in the freely-moving guinea-pig

95P **Sayer TJO, Hannon SD, Redfern PH & Martin KF** Diurnal variation in nerve terminal 5-HT autoreceptor function *in vivo*: effects of antidepressant treatment

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121P **Gray GA & Clozel M** ET-1 activates multiple receptor subtypes, including an inhibitory ETA receptor, in the rat fundic strip

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252P **Brunn G, Hey C, Reimann A, Wessler I & Racké K** Effects of alveolar macrophages on arachidonic acid (AA) metabolism in rat tracheal epithelial cells in primary culture

253P **Banner KH & Page CP** Comparison of the effect of the phosphodiesterase (PDE) III/IV inhibitor, zardaverine, administered acutely and chronically on pulmonary cell influx in ovalbumin sensitised guinea-pigs

254P **Underwood SL, Lee TH, Kemeny DM, Karlsson J-A & Raeburn D** The effect of ricin on IgE production, antigen-induced airway inflammation and airway hyperreactivity in the Brown Norway rat

255P **Krishek BJ, Xie X, Moss SJ & Smart TG** Functional modulation of recombinant $GABA_A$ receptors by activation of protein kinase C

256P **Wingrove PB, Wafford KA, Bain C, Kemp JA & Whiting PJ** Identification of a single amino acid on the β subunit of the $GABA_A$ receptor required for the modulatory actionof loreclezole

257P **Belelli D, Lambert JJ, Peters JA, Chen R, Reyes A & Lan NC** The functional properties of a *Drosophila* GABA subunit expressed in *Xenopus* oocytes

258P **Quirk K, Gillard N, Whiting PJ, Ragan CI & McKernan RM** Structure and pharmacological characterisation of δ-containing $GABA_A$ receptors in rat brain

259P **Gillard NP, Gu Z-Q, Rice K, Hadingham K, Cook J & McKernan RM** Substitution at position 8 of imidazo-benzodiazepine-6-ones is a determinant for $GABA_A$ α5-subtype activity

260P **Anderson SMP, Harrop JE, De Souza RJ & Cross AJ** The effects of differentiation of IMR32 cells with PGE_1 on $GABA_A$ receptor density

261P **Odber J, Cutler M & Moore MR** The effects of 5-aminolaevulinic acid and γ-aminobutyric acid on [^{3}H]-flunitrazepam binding to rat cerebral cortex membranes

262P **Jones RSG** GABAergic modification of 4-amino-pyridine-induced epileptiform activity in the rat entorhinal cortex

263P **Hassoni A, Wittwer R, Gray PTA & Bowery NG** Baclofen modulation of voltage-gated Ca^{2+} channels in rat cultured cerebrocortical neurones

264P **Houston AJ, Wong CLJ & Ebenzer IS** Baclofen inhibits water intake induced by systemic administration of the angiotensin converting enzyme (ACE) inhibitor, captopril

265P **Hoffmann S, Murray HE, Rantle C & Gillies GE** Investigations on the uptake and release of [^{3}H]GABA from perinatal hypothalamus in primary cell culture

266P **Davidson K & Gillies GE** Novel actions of a benzodiazepine and a barbiturate on somatostatin release from the perinatal hypothalamus in culture

267P **Cross AJ, Misra A, Sandilands AJ & Green AR** The effect of chlormethiazole on cGMP content of rat cerebellar slices

268P **Jackson HC** Hypothermic effects of NNC-711 and other GABA uptake inhibitors in mice

269P **Abdulla FA, Abu-Bakra MAJ, Calaminici M-R, Campbell IC, Stephenson JD & Sinden JD** Deficits in spatial learning are correlated with changes in sensitivity of frontal cortical neurones to acetylcholine and bicuculline in aged rats

270P **Hogg S & File SE** Quail selected for short or long tonic immobility responses differ in benzodiazepine receptor binding affinity: reflection of activity or fearfulness?

271P **Pratt JA & Brett RR** Increases in brain prepro-cholecystokinin messenger RNA levels in rats following acute administration of FG 7142

272P **Crawford S, Dawson GR & Tricklebank MD** Anxiolytic-like effects of amphetamine sulphate on the elevated plus maze

273P **Watson WP & Little HJ** The actions of the putative endogenous calcium channel ligand, palmitoyl carnitine, with acutely administered ethanol and in the ethanol withdrawal syndrome

274P **Watson WP & Little HJ** The effect of two dihydropyridine calcium channel antagonists on the distinctive convulsive components of ethanol withdrawal

275P **Kaura S, Young AMJ, Croucher MJ & Bradford HF** Release of neurotransmitter amino acids from basolateral amygdaloid complex *in vivo*

276P **Simpson PB, Challiss RAJ & Nahorski SR** Carbachol and *N*-methyl-D-aspartate evoke Ca^{2+} release from different intracellular stores in cultured cerebellar granule cells

277P **Bond A & Lodge D** Pharmacology of metabotropic glutamate receptor activation of rat spinal neurones *in vivo*

278P **Graham JL, Smith SE, Chapman AG & Meldrum BS** Effect of lamotrigine on microdialysate amino acid concentration in the striatum after focal cerebral ischaemia in the rat

279P **Nankai M, Nicolas C, Fage D & Carter C** Ifenprodil, eliprodil, magnesium, dextrorphan and polyamine spider toxins discriminate native NMDA receptor subtypes in the rat striatum

280P **Mentis GZ, Bull T, Moran L & Navarrete R** Somatic and dendritic degenerative changes induced by NMDA in neonatal rat flexor motoneurones *in vitro*

281P **MacGregor DG, Higgins MJ & Stone TW** Ascorbate or allopurinol protect against kainate-induced neurotoxicity

282P **Wood NI, Rothaul AL, Roberts JC & Hunter AJ** Comparison of effects of isoflurane versus halothane anaesthesia on lesion volume in rat model of focal cerebral ischaemia

283P **Milkowski NA, Wood NI, Patel S, Rothaul AL & Hunter AJ** Flunarizine fails to reduce lesion volume in a non-human primate model of focal cerebral ischaemia

284P **von Bussmann KA, Garey LJ & Jen LS** Differential effects of dizocilpine and flunarizine on the survival of retinal ganglion cells after injury

285P **Green AR & Cross AJ** The effect of chlormethiazole on the development of oedema following a cortical infarct

286P **MacDonald E, Haapalinna A & Heinonen E** Metabolites of selegiline provide protection against DSP-4 induced depletion of mouse frontal cortex noradrenaline levels

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288P **Alam AM & Starr MS** Do D_3 receptors control the limbic seizure threshold?

289P **Woodward R, Strange PG & Naylor LH** Mutation of serine residues 194 and 197 in the rat D_2 dopamine receptor affects the binding of antagonist drugs

290P **Presland JP & Strange PG** Expression of D_2 dopamine receptors in the yeast *S. Pombe*

291P **Lawlor M, Farrell CB, Dunne A & O'Boyle KM** Lack of effect of D_2 antagonists on the interaction between dopamine and D_1 dopamine receptors in rat and bovine striata

292P **Takada K, Baker S, Kumar R & Stephenson JD** Dopaminergic mechanisms in the entorhinal cortex alter dopamine efflux in the female rat nucleus accumbens and are modulated by oestrogen and progesterone

293P **Mason SL & Reynolds GP** Clozapine has elevated affinity for human frontal cortical dopamine D_2-like receptors

294P **Varty GB & Higgins GA** Involvement of dopamine D_2-like receptors in the modulation of prepulse-inhibition (PPI) in rats

295P **Black MD & Humphrey PPA** The effects of nitric oxide synthase inhibitors and oxyhaemoglobin on rat striatal dopamine overflow using fast cyclic voltammetry

296P **Chang W Y & Webster RA** OMD inhibition of L-dopa augmented dopamine release *in vitro*

297P **Saulskaya N & Marsden CA** Effect of MK-801 on dopamine release in the rat nucleus accumbens during a conditioned emotional response to a contextual cue

298P **Hartley JE, Forster EA & Fletcher A** The effects of WAY-100635, a potent and selective 5-HT receptor antagonist, on feeding and 8-OH-DPAT-induced feeding in the rat

299P **Gurling J, Ashworth-Preece MA, Dourish CT & Routledge C** Effects of acute and chronic treatment with the selective 5-HT_{1A} receptor antagonist WAY-100635 on hippocampal 5-HT release *in vivo*

300P **Brammer NT, Dunlop J, Lever D & Ennis C** PAPP, a 5-HT_{1D} receptor ligand, is not a 5-HT autoreceptor agonist or antagonist in the guinea-pig frontal cortex

301P **Roberts C, Watson J, Price GW & Jones BJ** Regional differences in the guinea-pig 5-HT terminal autoreceptor

302P **Reynolds GP, Mason SL, Meldrum A, De Keczer S, Parnes H, Eglen RM & Wong EHF** Characterization of the distribution of 5-HT_4 receptors in human brain using [^{3}H]GR113808

303P **Kennett GA, Bailey F, Piper DC & Blackburn TP** Effect of SB 200646A, a 5-HT_{2C} receptor antagonist, on two conflict models of anxiety

304P **Bourson A, Wanner D, Wyler R & Sleight AJ** A pharmacological study of the MCPP discriminative stimulus

305P **Graham S, Ho M-Y, Bradshaw CM & Szabadi E** Accelerated learning of a temporal discrimination following destruction of the ascending 5-hydroxytryptaminergic pathways.

306P **Rogers H, Abrams CJ & Kilpatrick GJ** *Ex vivo* binding of 5-HT_3 antagonists to 5-HT_3 receptors in the rat entorhinal cortex and colon

307P **Fone KCF & Scott M** Modulation of calcitonin gene-related peptide release from rat spinal cord slices

308P **Harder JA & Kelly ME** Effect of ondansetron on a water maze acquisition deficit produced by combined pCPA and scopolamine treatment

309P **Koshikawa N, Tomiyama K & Stephenson JD** Effects of acute and chronic treatments with tianeptine and paroxetine on 5-HTP-evoked changes in 5-HT content of rat hippocampal dialysates

310P **Lightowler S, Kennett GA, Wood MD, Brown AM, Glen A, Blackburn TP & Tulloch IF** Hypophagic effect of fluoxetine in rats is not mediated by inhibition of 5-HT reuptake or an agonist action at 5-HT_{2C} receptors

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313P **Gao B & Cutler MG** Effects of imipramine, mianserin and phenelzine on social behaviour in mice

314P **Esnaud H, Agnel M, Langer SZ & Graham D** Pharmacological characterization of the cloned human 5-HT transporter

315P **Parkins KJ, Cheng CHK, Costall B & Naylor RJ** Effect of 5-HT_3 receptor ligands on *in vivo* acetylcholine release from the rat hippocampus

316P **Sepúlveda MI & Lummis SCR** 5-HT_3 agonists can distinguish cloned 5-HT_3 receptors from NCB20 and NG108-15 cell lines

317P **Giles H, Lansdell SJ, Fox P, Lockyer M, Hall V & Martin GR** Characterisation of a 5-HT_{1B} receptor on CHO cells: functional responses in the absence of radioligand binding

318P **Stevens MC, Sangra M, Ritchie IM, Reidy MJ, Neilson FP, Kelly PAT, Kelly B, Dickson EM & Cursham MJA** Neocortical hyperaemia produced in rat by acute exposure to methylene-dioxymethamphetamine

319P **Heal DJ, Cheetham SC, Butler SA, Prow MR & Buckett WR** Evidence to demonstrate that postsynaptic α_2 adrenoceptors in the rat cortex are of the a_{2D} subtype

320P **Hudson AL, Husbands S, Lewis JW & Nutt DJ** Affinity and selectivity of BU224 and BU239 for rabbit brain non-adrenoceptor idazoxan binding sites (I_2-sites)

321P **Purkiss JR** Effect of incorporation of phosphatidyl-ethanol on release of noradrenaline from SH-SY5Y human neuroblastoma cells

322P **Kumari M, Cover PO, Poyser RH & Buckingham JC** Neuroendocrine responses to rolipram, denbufylline and 1,3-dicyclopropylmethyl-8-amino-xanthine (BRL 61063) in the rat

323P **Robinson KA, Hodgkiss JP & Kelly JS** The effects of 2-chloro-N^6-cyclopentyladenosine (CCPA) on spontaneous miniature excitatory postsynaptic potentials in rat hippocampal CA1 neurones

324P **Hosseinzadeh H & Stone TW** Modulation of post-synaptic adenosine sensitivity in calcium-free media in rat hippocampal slices

325P **Anderson R, Ray K, Rollins P, Sheehan MJ & Strong P** Functional characterisation of a human hippocampal adenosine A_1 receptor

326P **Kidd EJ, Michel AD & Humphrey PPA** Regional autoradiographic localisation of high affinity [^{3}H]α,β-methylene ATP binding sites in the rat brain

327P **Kirkpatrick KA & Richardson PJ** Role of cAMP in A_{2A} receptor mediated stimulation of [^{3}H]-ACh release from rat striatal synaptosomes

328P **Cheng CHK, Costall B, Naylor RJ & Parkins KJ** Differential *in vivo* acetylcholine release between hemispheres in the rat hippocampus

329P **Timothy C, Marshall JWB & Kelly ME** The effect of scopolamine on the acquisition of cocaine-induced conditioned place preference in the mouse

330P **Grant B, Balfour DJK & Struthers AD** The effects of captopril and its withdrawal on rat behaviour in 8-arm radial maze tests of learning and memory

331P **Ebenezer IS & Parrott RF** Intracerebroventricular (ICV) administration of the CCK_A receptor antagonist A70104 increases operant food intake in pigs

332P **Walsh DM, Beresford IJM & Hagan RM** Profile of GR159897, a non-peptide tachykinin NK_2 receptor antagonist, in a primate intruder response model

333P **Marshall FH, Smith GD, Turpin MP, Fallaize LA, Beresford IJM, Birch PJ & Hagan RM** Measurement of the CNS penetration of tachykinin NK_1 receptor antagonists in the gerbil using *ex vivo* binding and a scratching response

334P **Burford NT, Tobin AB & Nahorski SR** Agonist-stimulated Ins (1,4,5)P_3 accumulation and Ca^{2+} mobilization from intact and permeabilized CHO cells expressing recombinant muscarinic receptor subtypes

335P **Bigaud M, Bastian S & Miller RC** Proendothelin-1-induced phosphatidylinositol turnover in rat striatum involves a phosphoramidon-sensitive endothelin converting enzyme

336P **Smart D, Smith G & Lambert DG** Are G-protein beta/gamma subunits responsible for the stimulation of Ins(1,4,5)P_3 formation by μ opioids in SH-SY5Y cells?

337P **Taylor HM & Reynolds DJM** Opioid-induced emesis in the ferret

338P **Busquets X, Escriba PV, Sastre M & García-Sevilla JA** Chronic heroin/morphine administration decreases protein kinase C immunoreactivity in the human and rat brain

339P **Owen SM, Sturman G & Freeman P** Metoprine and nociception in the mouse

340P **Klamt JG, Follenfant R & Nakamura-Craig M** Intrathecal administration of lamotrigine induces analgesia in acute and chronic pain models in rats

341P **Wong CLJ** The effect of histamine H_2 receptor agonist and antagonist on restraint-induced antinociception in female mice is dependent on the time of drug administration

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344P **Brown JD, O'Shaughnessy CT, Kilpatrick GJ, Scopes DIC, Beswick P, Clitherow JW & Barnes JC** Characterisation of the specific binding of the histamine H_3 receptor antagonist radioligand [^{3}H]GR168320

345P **Clark EA & Hill SJ** Influence of sodium ions on the binding of thioperamide to histamine H_3 receptors in rat cerebral cortex

346P **Bischoff A, Farke W, Lewejohann K, Feth F, Rascher W & Michel MC** Ontogenesis of NPY content, NPY receptors and G-proteins in cerebral cortex of normotensive and hypertensive rats

347P **Bowman D, Smith W, McCormack A & Walker L** Affinity purification of synaptosomes using a biotinylated derivative of ω-conotoxin GVIA

348P **Kelly PAT, Ritchie IM & Arbuthnott GW** 7-Nitro indazole uncouples cerebral blood flow from metabolic demand in the conscious rat

349P **Aldridge CR & Collard KJ** The effect of depolarisation on arginine uptake by cerebellar and cortical synaptosomes

350P **Johnston HM & Morris BJ** Nitric oxide induces changes in MAP2 gene expression in hippocampal granule cells

351P **Bland-Ward PA, Pitcher A, Wallace P, Gaffen Z, Babbedge RC & Moore PK** Isoform selectivity of indazole-based nitric oxide synthase inhibitors

352P **Vellucci SV, da Costa AC, Parrott RF & Kendrick KM** Stress-related changes in the sheep induced by centrally-administered interleukin-1β: increases in plasma cortisol and mRNA for CRH and c-Fos in the paraventricular hypothalamic nucleus

353P **Mirtella A, Pozzoli G, Preziosi P & Navarra P** The effects of lipopolysaccharide and prostaglandins on immunoreactive interleukin-1α release from rat hypothalamic explants

354P **Widdowson PS, Trainor A & Lock EA** Subtle differences between the glutamate binding sites of cerebellar *vs* forebrain NMDA receptors

355P **Oblin A, Carter C & Schoemaker H** Pharmacological characterization of the binding of the glycine antagonist [^{3}H]5,7-dichlorokynurenate and its modulation by spermine

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357P **De Sarro GB, Bertorelli R, De Sarro A & Ongini E** Anticonvulsant effects of felbamate in DBA/2 mice: a possible involvement of NMDA and non-NMDA receptor complexes

358P **Knott NJ & Davies JA** L-arginine and glycine modulate N-methyl-D-aspartate (NMDA)-induced release of glutamate from rat striatal slices

359P **Barnes JM & Henley JM** Kainate modulation of [^{3}H]glutamate release from rat hippocampal synaptosomes

360P **Paul M, Philip J, McAllister M, Buckingham JC & Cowell AM** Effects of excitatory amino acids on growth hormone secretion from rat pituitary tissue *in vitro*

361P **Hutson PH, Bristow LJ, Thorn L & Tricklebank MD** The NMDA receptor antagonists (±)-CPP and CGS 19755 attenuate the behavioural and neurochemical effects of amphetamine in mice

362P **Kojima H, Covernton P, Sivilotti L, Gibb AJ & Colquhoun D** Relative agonist potencies at neuronal nicotinic receptors: a comparison between ganglionic and recombinant receptors

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365P **Wessler I, Härle P, Höhle Ch & Racké K** Modulation by muscarinic receptors of [^{3}H]acetylcholine release from isolated human bronchi: effect of indomethacin

366P **Kam KL, Pfaffendorff M & van Zwieten PA** Spontaneously hypertensive rats with diabetes: endothelium-dependent and -independent relaxation of isolated small arteries

367P **Pijl AJ, van der Wal AC, Mathy M-J, Kam KL, Hendriks MGC, Pfaffendorff M & van Zwieten PA** Streptozotocin-induced diabetes mellitus in spontaneously hypertensive rats: a pathophysiological model for the combined effects of hypertension and diabetes

368P **Davey NJ, Morrissey SE, Hastings LH & Laycock JF** The effect of *Phyllanthus sellowianus* extract in streptozotocin-diabetic rats

369P **Shafiee-Nick R, El-Metwaly M, Pyne N & Furman BL** Effects of isoenzyme-specific phosphodiesterase inhibitors on insulin secretion in the rat

370P **Lee K, Rowe ICM & Ashford MLJ** Pharmacological modulation of the trypsin modified K_{ATP} in the CRI-G1 insulin-secreting cell line

371P **Curwen JO, Johnson IR & Hatton R** Pioglitazone, an orally active anti-diabetic agent, decreases the vascular reactivity of obese Zucker rats but not that of spontaneously hypertensive rats

372P **Karasu Ç, Dewhurst M, Stevens EJ & Tomlinson DR** The effects of treatment with essential fatty acids and anti-oxidants on sciatic nerve dysfunction in streptozotocin-diabetic rats

373P **Dewhurst M, Stevens EJ, Karasu Ç & Tomlinson DR** Effects of aminoguanidine treatment on sciatic nerve laser Doppler flux and prostacyclin release from vascular tissues and sciatic nerves of control and streptozotocin-diabetic rats

374P **Lucas PD** Thermal sensitivity in streptozotocin-diabetic rats

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376P **Kirk JE, Rutherford RAD, Wharton J & Wilkins MR** Effect of experimental papillary necrosis on renal response to atrial natriuretic peptide and E-24.11 inhibition

377P **Abdi A & Johns EJ** Relationship between inhibition of angiotensin II production and acute renal failure during antihypertensive treatment in 2K2C Goldblatt hypertensive rats

378P **Clark K, Robertson MJ & Drew GM** Renal effects of the non-peptide angiotensin AT_1 receptor antagonist, GR138950

379P **Leeuwin RS, Zeegers A & van Wilgenburg H** Ca^{2+}-entry blockers modulate the inotropic response of the isolated rat heart to benzodiazepines

380P **Sun W, Wainwright CL & Parratt JR** The antiarrhythmic effect of ischaemic preconditioning is not prevented by inhibition of endothelium-derived nitric oxide in anaesthetized rats

381P **Pabla R & Curtis MJ** Failure of sodium nitroprusside to prevent reperfusion-induced ventricular fibrillation following brief ischaemia in rat isolated heart

382P **Lad N, Williams TJ & Booth RFG** Effect of Ro 25-3562, a selective leukotriene B_4 receptor antagonist, on neutrophil accumulation in the ischaemic/reperfused rabbit heart *in vitro*

383P **Hide E, Ney P, Thiemermann C & Vane JR** Prostaglandin E_1 and prostaglandin E_0 reduce infarct size in a rabbit model of acute myocardial ischaemia and reperfusion

384P **Grocott-Mason RM, Lewis MJ & Shah AM** Nitric oxide selectively enhances relaxation in the intact ventricle

385P **Patel VC, Woolfson RG, Neild GH & Yellon DM** Inhibition of nitric oxide reduces infarct size in the rabbit isolated heart through adenosine release

386P **Workman AJ, MacKenzie I & Northover BJ** The role of ATP-sensitive potassium (K_{ATP}) channels in cardiac arrhythmias in rat isolated hearts

387P **Heath BM & Terrar DA** Parallel activation of K_{ATP} and reduction of delayed rectifier current by cromakalim in guinea-pig ventricular cardiac myocytes

388P **Smith K & Docherty JR** Further investigations of the prejunctional α_2-adrenoceptors of rat atrium

389P **Du XY, Schoemaker RG & Saxena PR** Different inotropic responsiveness in human atrial and ventricular trabeculae

390P **Zhou BY & Lab MJ** 4-acetamide-4′-isothio-cyanatostilbene-2,2′-disulphonic acid inhibits stretch-induced ventricular premature contractions in isolated guinea-pig hearts

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392P **Dubois EA, Janssen A, Batink HD, van Royen EA, Pfaffendorff M & van Zwieten PA** [^{123}I]4-iodo-dexetimide as a radioligand for the imaging of cardiac muscarinic receptors

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396P **Gelzer AM, Braun E, Szwarc RS & Ball HA** Haemodynamic effects of the NO donor SIN-1 in the anaesthetised mini-pig: quantification of myocardial oxygen consumption by pressure-volume area

397P **Spiers JP, Donaghy KM & Scholfield CN** Interactions between phorbol-12,13-dibutyrate and lignocaine in sheep Purkinje fibres

398P **Chlopicki S, Slawinski M, Gryglewski R & Parratt JR** Mediators involved in the antiarrhythmic effect of ischaemic preconditioning in guinea-pig isolated hearts

399P **Vegh A, Papp J Gy, Szekeres L & Parratt JR** Blocking adenosine receptors fails to modify the pronounced antiarrhythmic effect of ischaemic preconditioning in the canine myocardium

400P **Dionisotti S, Conti A, Sandoli D, Zocchi C, Gatta F & Ongini E** *In vitro* pharmacological profile of selected adenosine A_2 antagonists

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402P **Wagner H, Milavec-Krizman M, Gadient F, Menninger K, Schoeffter P & Fozard J** Species-dependent cardiovascular effects of SDZ WAG 994, a selective adenosine A_1 receptor agonist

403P **Ford WR & Broadley KJ** Involvement of different calcium sources in the inhibition of phenylephrine contractions of guinea-pig aorta by NECA

404P **Jarvis JE, Reeves JJ, Sheehan MJ & Strong P** Not all adenosine receptor blocking drugs augment adenosine A_1-receptor-mediated contractions in rat colon

405P **Trezise DJ, Bell NJ & Humphrey PPA** Ionic dependence of depolarising responses evoked by α,β-methylene ATP in the rat isolated vagus nerve

406P **Khakh BS, Kennedy I & Humphrey PPA** Relative agonist potencies at P_2 purinoceptors in rat and guinea-pig vas deferens

407P **Patel V & Boarder MR** Both P_{2Y} purinoceptors and nucleotide receptors stimulate arachidonic acid release from cultured aortic endothelial cells

408P **Connolly GP, Harrison PJ & Stone TW** α,β-methylene ATP but not uridine-5′-triphosphate-evoked depolarisations of the rat superior cervical ganglion are antagonised by a P_{2X}-purinoceptor antagonist

409P **Andriantsitohaina R, André A, Lagaud GJL & Stoclet JC** Modulation by SIN-1 of the responses to ATP and to noradrenaline in rat isolated mesenteric resistance arteries

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412P **Willars GB & Nahorski SR** Calcium store depletion may underlie muscarinic-induced inhibition of intracellular calcium responses to bradykinin in SH-SY5Y cells

413P **Grantham CJ, Bowman D, Miller RJ, Brust PF, Ellis SB, Harpold MM & Bleakman D** Pharmacological characterisation of the human N-type calcium channel in transfected HEK 293 cells

414P **Pérez-Vizcaíno F, Fernández del Pozo B & Tamargo J** Voltage- and time-dependent inhibitory effects of propafenone and quinidine in vascular smooth muscle

415P **Beech DJ** Inhibition of voltage-dependent Ca-current by imidazole derivatives in intestinal smooth muscle cells from the guinea-pig

416P **Masood N & Naylor IL** The *in vitro* reactivity of fascia from the rat and guinea-pig to calcium ions and mepyramine

417P **McMillan MR, Snook J, Garland CJ & Murphy TV** Sources of Ca^{2+} in noradrenaline and adenosine 5'-triphosphate (ATP)-evoked contraction of rabbit proximal saphenous artery

418P **Lang D, Shah AM & Lewis MJ** The ACE inhibitor ramiprilat augments bradykinin-induced changes in $[Ca^{2+}]_i$ in both bovine aortic and endocardial endothelial cells

419P **Szabó Cs, Thiemermann C, Wu CC & Vane JR** Dihydropyridine calcium channel agonists and antagonists inhibit the induction of NO synthase and prevent the inhibition of cellular respiration caused by endotoxin in J774.2 macrophages

420P **Boddeke HWGM & Seuwen K** Cloned human parathyroid hormone receptors mobilise cytosolic calcium through a heparin-insensitive mechanism

421P **Garcha RS & Hughes AD** Evidence for a single intracellular calcium store mobilised by noradrenaline and caffeine in rat isolated mesenteric arteries

422P **Boot JR & O'Brien A** The effect of calcium channel antagonists on the mammalian neuromuscular junction

423P **Musgrave IF, Seifert R & Schultz G** Matiotoxin stimulates calcium influx in HL-60 cells through a pathway distinct from receptor-activated non-selective cation channels

424P **Mantel P, van Weeren-Kramer J, Leeuwin RS & van Wilgenburg H** Enforcement of tetanic contraction prevails in biphasic effect induced by adinazolam

425P **Suzuki Y, Yagami Y & Fujimoto S** Relaxation responses of human uterine and spontaneously hypertensive rat femoral arteries to parathyroid hormone, histamine and acetylcholine

426P **Randall MD, Ujiie H & Griffith TM** Exogenous L-arginine reverses impairment of EDRF-dependent collateral perfusion in a rabbit model of hypercholesterolaemia

427P **Calder JA, Schachter M & Sever PS** Indapamide binding in guinea pig vascular smooth muscle

428P **Shahidullah M & Wilson WS** Effects of atriopeptin on aqueous humour formation and on cyclic GMP in cultured ciliary epithelium of the bovine eye

429P **Liu JT, Paul W & Page CP** Effect of thrombin inhibitors, anti-coagulants and a fibrinolytic agent on thrombin-induced cerebral embolism in rabbits

430P **Allport JR, Donnelly LE, Boyd RS, Ray KP & MacDermot J** Vitamin K inhibits chemotaxis and actin polymerization in human polymorphonuclear neutrophil leukocytes

431P **Scott HJ, Marshall K & Senior J** A preliminary study on the effects of bradykinin and related compounds on isolated mouse uterus

432P **Kennedy F, Marshall K & Senior J** Characterisation of the thromboxane (TP) receptor population in isolated mouse uterus

433P **Abbas F, Marshall K & Senior J** The effect of Des-Arg^9 (Leu^8) BK (a B_1 receptor antagonist) on the actions of bradykinin (BK) and related compounds on isolated human myometrium

434P **Grossman CJ, Trevethick MA, Wiseman J, Bountra C & Birch PJ** Bacterial lipopolysaccharide induces *de novo* synthesis of cyclo-oxygenase activity in freshly isolated human mononuclear cells

435P **Al-Hafidh J, Mills S, Lampe D, Liu C, Potter BVL & Westwick J** *Myo*-inositol-1,4,6-trisphosphorothioate: an inhibitor of inositol trisphosphate-induced $^{45}Ca^{2+}$ release

436P **Leboeuf J, Blanchon R, Tardif S, Winslow E & Massingham R** Collagen-induced ST segment elevation in anaesthetized rats: effects of aspirin, dipyridamole and calcium antagonist drugs

DEMONSTRATIONS

437P **Brown ER, Dewhurst DG & Williams AD** An interactive computer simulation of the squid giant axon preparation for teaching undergraduate students

438P **Dewhurst DG, Brain S & Ullyott RT** A computer simulation of experiments to demonstrate the effects of pharmacological agents on the cutaneous inflammatory response in the anaesthetised rabbit

439P **Whelpton R** Demonstration of a Windows-based programme for multiple choice questions

440P **Bowen WP & Jerman JC** INFLEXION: automated analysis of radioligand binding data with Microsoft Excel

CONTRIBUTION BY TITLE ONLY

441P **Starkey SJ & Skingle M** 5-HT_{1D} autoreceptors modulate stimulated 5-HT release in the guinea-pig dorsal raphé nucleus

ORAL COMMUNICATIONS

In oral communications with more than one author, the first author is the one who intended to present the work

442P **Dockrell MEC, O'Kane KPJ, Haynes WG, Williams BC & Webb DJ** Platelet-derived nitric oxide in hypertensive and normotensive control subjects

443P **De Kimpe SJ, Thiemermann C & Vane JR** Lipoteichoic acid, a cell wall component of gram positive bacteria, causes hypotension and induction of nitric oxide synthase in anaesthetised rats

444P **Bryant CE, Allcock GH, Warner TD & Vane JR** Comparison of the cardiovascular responses to chronic and acute treatment with L-nitroarginine methyl ester in the rat

445P **Pires J, Futuro-Neto HA, Trindade JD & Ramage AG** An investigation of the interaction between L-NAME and angiotensin II on blood pressure and renal sympathetic nerve activity in α-chloralose anaesthetized rats

446P **Barker JE, Anderson J, Treasure T & Piper PJ** Effects of prostaglandins on stimulated release of nitric oxide by angiotensin II from human saphenous vein

447P **Chatterjee PK, Nicholson G, Jardine A, Hawksworth GM & McLay JS** Attenuation of cytokine-stimulated nitric oxide production by h-ANP in human renal proximal tubular cells is not mediated by cyclic GMP

448P **Ghelani A, Wallace A & Boughton-Smith NK** Involvement and cellular source of induced nitric oxide synthase in zymosan peritonitis in the rat

449P **Laszlo F, Whittle BJR & Moncada S** Protective actions of the nitric oxide donor S-nitroso-glutathione or an anti-neutrophil antibody in acute microvascular injury in rat intestine

450P **Constantin-Teodosiu D, Greenhaff PL, Gardiner SM & Bennett T** The effect of chronic ingestion of a nitric oxide synthase inhibitor on cardiac tissue energy metabolism

451P **Cockroft JR, Chowienczyk PJ, Brett SE & Ritter JM** Effect of N^G-monomethyl-L-arginine on vasodilator responses to bradykinin and substance P in the human forearm vascular bed

452P **Chowienczyk PJ, Cockroft JR & Ritter JM** Nitric oxide independent vasodilatation by acetylcholine in human forearm vasculature is selectively potentiated by edrophonium

453P **Tan KKC, Brown MJ, Hargreaves RJ, Shepheard SL, Cook DA & Hill RG** Calcitonin gene-related peptide (CGRP) as an endogenous vasodilator: *in vivo* immunoblockade studies with an anti-CGRP monoclonal antibody and its Fab' fragment

454P **Dowell FJ, Hamilton CA, Jardine EJ & Reid JL** Effects of a xanthine oxidase/hypoxanthine free radical/reactive oxygen species on vascular function in rabbit aortae

455P **Moulson A, Wan BYC & Pearce FL** Role of antioxidants in the H_2O_2 inhibition of histamine release from rodent mast cells

456P **Peakman M-C & Hill SJ** Endogenous expression of histamine H_1-receptors functionally coupled to phosphoinositide hydrolysis in C6 glioma cells: regulation by cyclic AMP

457P **Koenig JA & Edwardson JM** Kinetic analysis of muscarinic ACh receptor trafficking in NG108-15 cells

458P **Harriss DR, Marsh KA, Birmingham AT & Hill SJ** Muscarinic M_3 receptor-mediated phosphoinositide hydrolysis in human cultured detrusor smooth muscle cells

459P **Kerr PM, Hillier K, Wallis RM & Garland CJ** Pharmacological characterisation of muscarinic receptors mediating contraction of human colonic smooth muscle

460P **Eglen RM, Reddy H & Watson N** Role of post-junctional muscarinic M_2 receptors in the control of guinea-pig tracheal smooth muscle tone

461P **Herbert CA, Omari TI, Sparrow MP & Robinson C** Human eosinophils and recombinant matrix metalloproteinase-2 increase permeability of the bovine and porcine bronchial mucosa

462P **Hall IP & Pearl G** Inhibition of human airway smooth muscle proliferation by dexamethasone

463P **Macmillan S, Sheridan RD & Patmore L** Comparison of the effects of NS-004 and SCA40 on the activity of single maxi-K^+ channels in bovine tracheal smooth muscle cells

464P **Zhang H & Bolton TB** Intracellular GDP, metabolic inhibition and pinacidil activate glibenclamide-sensitive K-channels in smooth muscle cells of rat mesenteric artery

465P **Wickenden AD & Prior H** Effect of the potassium channel openers cromakalim, pinacidil and RP49356 on the guinea-pig isolated diaphragm

466P **Benton DCH, Athmani S, Roxburgh CJ, Shiner MAR, Haylett DG, Ganellin CR & Jenkinson DH** Effects of cetiedil and its analogues on the Ca^{2+}-activated K^+ permeability of rabbit erythrocytes and on levcromakalim-stimulated $^{86}Rb^+$ efflux from rat aorta

467P **Jones AG, Dunn PM & Haylett DG** Inhibitors of G-protein-activated K^+ conductances in rat atrial myocytes and guinea-pig submucous plexus neurones

468P **Isaac L, Cook SJ & Small RC** The effect of Rb^+-substituted Krebs solution on the electrical and mechanical activity of guinea-pig isolated trachealis muscle and on tracheal responses to relaxant agents

469P **Cook SJ, Archer K, Martin A, Buchheit KH, Fozard JR & Small RC** Evidence that K^+-channel opening is not important in the tracheal relaxant action of SCA 40

470P **McKean J & MacDonald A** Analysis of the propranolol-sensitive relaxation to isoprenaline in rat distal colon

471P **Oriowo MA** Atypical β-adrenoceptors in a vascular smooth muscle preparation

472P **Dando SB, Jordan D & Ramage AG** Evidence that buspirone potentiates the vagal bradycardia induced by upper airway stimulation in anaesthetised rabbits

473P **Byrne C & Baxter GS** Further evaluation of putative 5-HT_{2A} receptors mediating relaxation of guinea-pig trachea

474P **Razzaque Z, Longmore J & Hill RG** Differences in the effects of ketanserin on 5-HT-receptor-mediated responses in rabbit saphenous vein and guinea-pig jugular vein

475P **Murphy OE & Baxter GS** 5-HT_{2B} receptors mediate contraction of rat stomach fundus circular muscle

476P **Baxter GS** Variability in 'effective receptor reserve' at putative 5-HT_{2B} receptors in rat stomach fundus

477P **Ellis ES, Byrne C, Murphy OE & Baxter GS** 5-HT_{2B}-like receptors mediate endothelium dependent relaxation of rat jugular vein

478P **Tilford NS & Baxter GS** The actions of ketanserin and ritanserin on contractile responses to sumatriptan in rabbit basilar artery

479P **Shaw LA & Coker SJ** Profound reduction in platelet responses after combined administration of 5-HT_2 and thromboxane antagonists

480P **Mistry R, Prabhu G & Challiss RAJ** Effects of the putative metabotropic glutamate receptor antagonist L-2-amino-3-phosphonopropionate on phosphoinositide turnover in neonatal rat cerebral cortex

481P **Rabbani M & Little HJ** Effects of chronic ethanol treatment on AMPA binding sites in cerebral cortex

482P **Obrenovitch TP, Urenjak J, Richards DA, Swayne GTG & Symon L** Intracerebral microdialysis combined with recording of extracellular field potential: a novel method for the investigation of depolarising drugs *in vivo*

483P **Maione S, Rossi F, Fowler LJ & Whitton PS** AMPA receptor stimulation increases 5-HT release in rat striatum and hippocampus *in vivo*

484P **Lawrence CB & Rothwell NJ** Interleukin-1 receptor antagonist inhibits NMDA and AMPA receptor-induced brain damage in the rat

485P **McGivern JG, Sheridan RD & Patmore L** The neuroprotective agent, lifarizine (RS-87476), inhibits sodium currents via an interaction with the slowly inactivating state of the channel

486P **Patel TR, McAuley MA & McCulloch J** The actions of Bosentan, an endothelin receptor antagonist, on cerebral arterioles *in situ*

487P **Qume M, Zeman S, Stratmann GC, Worth C, Francis PT, Procter AW & Bowen DM** A neurochemical study of non-Alzheimer dementia

488P **Skingle M, Sleight AJ & Feniuk W** Microdialysis study investigating the effects of GR127935, a potent 5-HT_{1D} receptor antagonist, on cortical levels of 5-HT in the guinea-pig

489P **Roberts C, Thorn L, Price GW, Middlemiss DN & Jones BJ** Effect of the selective 5-HT_{1D} receptor antagonist, GR 127935, on *in vivo* 5-HT release, synthesis and turnover in the guinea-pig frontal cortex

490P **Rick CE & Lacey MG** Does 5-HT2C receptor activation cause excitation of substantia nigra pars reticulata neurones in rat brain slices?

491P **Needham PL, Skill MJ, Kettle M & Heal DJ** Attenuation of catalepsy by the 5-HT_{1A}/5-HT_7 agonist, 8-OH-DPAT, is mediated by postsynaptic 5-HT receptors

492P **Lima L, Laporte AM, Gaymard C, Lanfumey L, Mocaër E & Hamon M** *In vitro* and *in vivo* labelling of central 5-HT_{1A} receptors with a new selective agonist radioligand [^{3}H]S 14506

493P **Battistini B, O'Donnell LJD, Warner TD, Fournier A, Farthing MJG & Vane JR** Characterisation of an endothelin-converting enzyme activity in the isolated gallbladder of the guinea-pig

494P **Peter MG & Davenport AP** [^{125}I]PD151242 as an endothelin ET_A-selective radioligand in the human, rat and porcine heart

495P **Maguire JJ, Kuc RE, O'Reilly GE & Davenport AP** Characterisation of vasoconstrictor endothelin receptors in human isolated renal artery and vein

496P **Wellings RP, Corder R & Vane JR** Endotoxin induces renal vasoconstriction in the anaesthetised rat: effect of the non-selective endothelin receptor antagonist, PD 145065

497P **Bate G, Luheshi GN, Brouwer S, Dascombe MJ, Rothwell NJ & Hopkins SJ** IL-1 receptor antagonist inhibits the endotoxin-induced rise in plasma IL-6 in the rat

498P **Lal H, Woodward B & Williams KI** Pulmonary vascular and bronchial actions of endothelins and sarafotoxin 6c in the rat isolated perfused lung

499P **Corder R, Findlay S, Khan N, Firth JD & Vane JR** Adenosine triphosphate, adenosine and 2-chloroadenosine inhibit endothelin-1 release from cultured endothelial cells

500P **Ralevic V, Hoyle CHV & Burnstock G** Pivotal role of phosphate chain length as a determinant of vasoconstrictor or vasodilator activity of adenine nucleotides in the rat mesenteric arterial bed

501P **Rubino A & Burnstock G** Contractile actions of pyrimidine and purine nucleotides in the rat pulmonary vascular bed

502P **Trezise DJ, Bell NJ & Humphrey PPA** P_2 purinoceptor antagonist effects of pyridoxal-5-phosphate in the rat vagus nerve and guinea-pig aorta

503P **Kelley AS & Hollingsworth M** Evidence that ATP and β,γ-methylene ATP produce relaxation of guinea-pig trachealis via an action involving P_1 purinoceptors

504P **Wainwright CL & Kang L-P** The effect of chronic pretreatment on the antiarrhythmic and haemodynamic effects of the A_1-adenosine agonist, R-PIA, in rat isolated hearts

505P **Patel M, Sheehan MJ & Strong P** Cardiovascular effects of GR79236, APNEA and CGS21680 in the anaesthetised rat

506P **Rose S, Hindmarsh JG, Silva MT, Pitcher A, Aislaitnor G, Moore PK, Gorrod JW, Jenner P & Marsden CD** The novel monosodium salt of 7-nitroindazole inhibits brain nitric oxide synthase and elevates striatal dopamine efflux *in vivo*

507P **Black MD, Matthews EK, Butler AR & Humphrey PPA** The effects of a photosensitive nitric oxide donor on dopamine efflux measured by fast cyclic voltammetry in the rat striatum

508P **Saywell KL, Bristow LJ, Landon L & Tricklebank MD** Effect of the putative dopamine D_3 receptor agonist, (+)-PD 128907, on prepulse inhibition of acoustic startle responding in the rat

509P **Watson WP & Little HL** Two calcium channel antagonists, nitrendipine and felodipine, exhibit opposite actions against the *in vivo* effects of the potassium channel blocker 4-aminopyridine

510P **Micklethwaite AV & Pleuvry BJ** The effect of propofol on epileptiform activity induced by zero magnesium in the cortical slice *in vitro*

511P **Atcheson R, Hirst RA, Rowbotham DJ & Lambert DG** The effect of halothane on [^{3}H]noradrenaline release from SH-SY5Y human neuroblastoma cells

512P **Ogilvy HV, Graham DI & Stone TW** Kainate-evoked purine release from rat hippocampus

513P **Michel AD, McGuire C, Grahames CBA & Humphrey PPA** Differences between the high affinity [^{3}H]α,β-methylene ATP binding sites in rat vas deferens and rat brain

514P **Croning MDR, Zetterström TSC & Newberry NR** Adenosine receptor antagonists attenuate the $[K^+]_e$ increase during hypoxia in the rat hippocampus *in vitro*

515P **Longmore J, Fong TM & Hill RG** The susceptibility of agonist-evoked responses to antagonism by (±) CP96345 and RP67580 differs for human and rat cloned NK_1 receptors

516P **Gardner CJ, Bountra C, Bunce KT, Dale TJ, Jordan CC, Twissell DJ & Ward P** Anti-emetic activity of neurokinin NK_1 receptor antagonists is mediated centrally in the ferret

517P **Traynor JR & Nahorski SR** Opioid-mediated stimulation of [^{35}S]GTPγS binding in SH-SY5Y human neuroblastoma cells

518P **Sitamze J-M, Wermuth CG & Martin RJ** The arylyaminopyridazine-GABA derivative NCS281-93 is a potent GABA antagonist in *Ascaris suum*

519P **Szabó Cs, Perretti M, Thiemermann C, Flower RJ & Vane JR** Effect of interleukin-4 on interleukin-1 induced neutrophil migration and on endotoxin-induced nitric oxide production in macrophages and neutrophils

520P **Foster AP, McCabe P, Sanjar S & Cunningham FM** Interleukin-5 induces the adhesion of equine eosinophils to fibronectin-coated plastic

521P **Harris JG, Perretti M & Flower RJ** Role of endogenous corticosterone in platelet-activating factor-induced neutrophilia in the mouse

522P **Griesbacher T, Sutliff RL & Lembeck F** Inhibition of inflammatory and algesic effects of an extract from *Porphyromonas gingivalis* by the bradykinin antagonist, icatibant (Hoe 140)

523P **Lydford SJ & McKechnie KCW** Characterisation of the prostaglandin receptors located on the rabbit isolated saphenous vein

524P **Ward PS, Vetterlein O, Bodmer M, Boden T & Foulkes R** Monoclonal antibodies: effect of multiple dosing on reducing immunogenicity

525P **Gardiner SM, Kemp PA & Bennett T** Effects of N-acetyl-5-hydroxytryptamine on regional haemodynamic responses to chronic endotoxaemia in conscious rats

526P **Ramage AG & Wyllie MG** Effects of doxazosin and terazosin on inferior mesenteric nerve activity, spontaneous bladder contraction and blood pressure in anaesthetised cats

527P **Soares-da-Silva P & Pinto-do-Ó PC** The cyanine derivative, 1,1-diethyl-2,4-cyanine (decynium 24) is a potent inhibitor of the renal tubular uptake of L-DOPA

528P **Cunningham SMC, Mihara S & Lees GM** Evidence that presynaptic modulation by neuropeptide Y of inhibitory transmission in submucous ganglia of guinea-pig caecum is mediated by Y_2-receptors

529P **Feniuk W & Humphrey PPA** Inhibitory effect of somatostatin in mouse vas deferens is insensitive to pertussis toxin treatment

POSTER COMMUNICATIONS

530P **Soares de Moura R & de Lemos Neto M** Effect of cromakalim on the contractility of the isolated human ureter

531P **Grant TL, Wickenden AD, Grimwood S, Todd MH, Gibson KH, Edwards G, Weston AH & Russell NJW** ZM172405 evokes smooth muscle relaxation via a K+ channel opener mechanism

532P **Criddle DN & Soares de Moura R** Inhibition of the vasorelaxant effects of levcromakalim and P1060 by rubidium in human saphenous vein

533P **Bruyninckx C, Steinmetz R, Chatelain P, Gautier P & Manning A** SR 47063, a new K^+ agonist: effect on reperfusion injury in comparison to cromakalim in isolated, perfused rat hearts

534P **McHarg A, Holland VM & Miller NM** Comparison of the *in vitro* activity of SCA40 with levcromakalim, BRL 55,834 and salbutamol in guinea-pig airways

535P **Isaac L & Small RC** The effects of some K^+-channel inhibitors on the actions of spasmogens in guinea-pig isolated trachealis muscle

536P **Sadraei H, Large BJ & Beech DJ** Potassium channel and calcium channel currents and their inhibition by glibenclamide in smooth muscle cells isolated from guinea-pig seminal vesicle

537P **Edwards JS & Ritter JM** Interactions between cytoplasmic pH and Ca^{2+} on thromboxane synthesis in human platelets

538P **Uenishi H, Liu JT, Itoh H, Paul W & Page CP** Effects of Hirulog and sodium nitroprusside plus recombinant tissue plasminogen activator on cerebral thromboembolism in rabbits

539P **Buckley C, McManus D, Bruckdorfer KR & Jacobs M** Hydroperoxy- and hydroxy-derivatives of arachidonic acid inhibit endothelium-dependent relaxation in rabbit isolated aorta

540P **Piotrowski W, Schryve L, Nagi S &Simon MC** Effects of the nitric oxide synthase inhibitors N^{ω}-nitro-L-arginine methyl ester, 7-nitro-indazole and related nitroindazoles on non-adrenergic, non-cholinergic responses of guinea-pig isolated taenia caeci

541P **Wu C-C, Chen S-J, Szabó Cs, Thiemermann C & Vane JR** Activation of soluble guanylyl cyclase by a factor other than nitric oxide or carbon monoxide contributes to the vascular hyporeactivity to endotoxin in the rat aorta

542P **Wright IK, Amirchetty-Rao S & Kendall DA** Forskolin potentiates both SNP- and ANP-stimulated cGMP accumulation in porcine isolated palmar lateral vein

543P **Soares de Moura R, Ferraz MR & Santos R** Effect of L-NAME on sexual behaviour in the male rat

544P **Soares-da-Silva P, Pestana M, Vieira-Coelho MA, Fernandes MH & Albino-Teixeira A** Enhanced renal dopaminergic activity during long-term nitric oxide synthase inhibition in the rat

545P **Hoare S & Strange PG** Allosteric regulation of the rat D_2 dopamine receptor by amiloride analogues

546P **Vile JM & Strange PG** Investigation of dopamine receptors on the peripheral blood lymphocytes of healthy volunteers

547P **Piper IT, Thompson DG & Weston AH** The role of acetylcholine and prostaglandin release in the action of hydrogen peroxide on guinea-pig distal colon

548P **Battistini B, Warner TD & Vane JR** Effects induced by linear peptide antagonists selective for ETA receptors: a study in different smooth muscle preparations

549P **Kent A & Keenan AK** Evidence for signalling by endothelin ET-A and ET-B receptors in bovine pulmonary artery smooth muscle cells

550P **Smith CFC, Baker C, Redmond L, Stoggall S, Wright N & Wilson C** Are different antagonist pA_2 values against endothelin-1 and endothelin-3 due to different ET_A receptor subtypes in guinea-pig, rat and rabbit vascular tissues?

551P **Harrison V, Khan N, Corder R & Vane JR** Inhibition of endogenous synthesis of endothelin-1 in cultured human and bovine endothelial and human epithelial cells by phosphoramidon and leupeptin

552P **Maguire JJ, Kuc RE, O'Reilly G & Davenport AP** Potency of the novel orally active endothelin antagonist Ro46-2005 for endothelin receptors in human vascular smooth muscle

553P **Slee S-J, Heys C & Wilson C** Effects of endothelin antagonists on vasoconstrictor and vasodilator responses to the ET_B agonist BQ3020 in pithed rats

554P **Davenport AP, Kuc RE, Hoskins SL & Karet FE** [^{125}I]PD151242 is selective for endothelin ET_A receptors in human kidney and localises to the vasculature

555P **Smith PW, McQueen DS & Webb DJ** The effect of cooling on the response to endothelin-1 in isolated rat mesenteric resistance arteries

556P **Haghighi MK & Pollock D** The possible involvement of nitric oxide in desensitisation to 5-HT in rat pulmonary artery rings

557P **Quinn P, Aston FA, Addley N, Halfpenny P & Wallis RM** The cardiovascular effects of 5-HT_{1A} selective compounds: antagonist studies with WAY-100,135

558P **Borman RA & Burleigh DE** Heterogeneity of 5-HT receptors in human sigmoid colon

559P **Hegde SS, Ku P, Lai K & Eglen RM** 5-HT_4 receptor-mediated contraction of colon in the anaesthetized guinea-pig

560P **Tuladhar BR, Costall B & Naylor RJ** Evidence of 5-HT receptor involvement in the facilitation of the peristaltic reflex in the marmoset ileum

561P **Shaw AM, Brown C, Elbishti W, Pollock D & McGrath JC** The effects of 5-HT on total cGMP content and perfusion pressure of the isolated rat lung

562P **Gardiner SM, Kemp PA & Bennett T** Regional haemodynamic effects of adrenomedullin in conscious rats

563P **Metcalfe M, Bushfield M, Naylor AM & Templeton D** Tachykinin-activated bladder contractions in the anaesthetised guinea-pig

564P **Hughes SJ & Hollingsworth M** Does relaxin inhibit myometrial mechanical activity via a cAMP-dependent pathway in rat uterus?

565P **Scott HJ, Marshall K & Senior J** The effect of Hoe-140 on the response of the isolated mouse uterus to bradykinin and related compounds

566P **Furukawa K, Wass N, McKinney SJ, Chapple CR, Smith D, Rosario DJ, Uchiyama T & Chess-Williams R** α_1-adrenoceptor subtype mediating contractile responses of the human vas deferens

567P **Murphy TV & Garland CJ** Noradrenaline, but not 5-hydroxytryptamine, stimulates accumulation of inositol (1,4,5)-trisphosphate in rabbit basilar artery

568P **Roberts BR & Shaw JS** Characterization of α_2-adrenoceptor subtype binding in transfected CHO cells

569P **Palea S, Rimland JM, Corsi M & Trist DG** Evidence for the presence of an atypical prejunctional NPY-Y_1 receptor in rabbit isolated vas deferens: comparison with the rat vas deferens

570P **Gupta P & Roberts LA** Characterisation of a prejunctional H_3 receptor in the dog isolated electrically-stimulated saphenous vein

571P **McDonald RL, Peers C & Vaughan PFT** Inhibitory effects of muscarinic receptor activation on K^+-evoked [^{3}H]noradrenaline release from the human neuroblastoma SH-SY5Y

572P **Coombes JE, Hughes AD & Thom SAMcG** Myogenic tone is associated with increased intracellular calcium in rat isolated mesenteric and cerebral small arteries

573P **Quinn P & Wallis RM** UK-84,149: a potent and gut-selective spasmolytic in the anaesthetised dog

574P **Wallis RM, Alker D, Burges RA, Cross PE, Greengrass PM, McIntyre P & Quinn P** UK-84,149: a novel gut-selective spasmolytic with calcium antagonist activity

575P **Banks A, Carrington AL & Tomlinson DR** The motor nerve conduction deficit is attenuated in streptozotocin-diabetic rats by treatment with inositol 1,2,6-trisphosphate without an effect on nerve blood flow

576P **Fatehi M, Rowan EG & Harvey AL** Characterization of the effects of depolarizing phospholipases A_2, myotoxin a and ouabain on the perineural waveforms recorded from mouse motor nerve endings

577P **Heath BM & Terrar DA** The influence of electrode chloride concentration on the delayed rectifier potassium current and on the blocking action of compound II and dofetilide

578P **Dainty IA, Pollard CE, Roberts SM, Franklin M, McKechnie KCW & Leff P** Evidence for subdivision of P_{2u}-purinoceptors based on suramin sensitivity

579P **Manning A, Hodeige D, Richard J, Heyndrickx J-P & Chatelain P** SR33589, a new amiodarone-like anti-arrhythmic agent: electrophysiological effects in dogs

580P **Meester BJ, Welsh NJ, Shankley NP, Meijler FL & Black JW** Classification of adenosine A_1 receptors by agonist potency orders in the sinoatrial and atrioventricular nodes of the guinea-pig

581P **Hu DE & Fan T-PD** Suramin inhibits the angiogenic activity of vascular endothelial growth factor *in vivo*

582P **Meester BJ, Welsh NJ, Shankley NP, Meijler FL & Black JW** Potentiating interaction between adenosine and 5-N-ethylcarboxamidoadenosine in the guinea-pig atrioventricular node

583P **Gould J, Bowmer CJ & Yates MS** Renal vascular reactivity in acute renal failure

584P **Amin F, Tatchum Talom R, Niederhoffer N, Guillou J & Atkinson J** Improvement of cardiac function following chronic antihypertensive treatment of spontaneously hypertensive rats with captopril plus hydrochlorothiazide

585P **Tran Ngoc Phuong N, Robert A, Atkinson J & Capdeville-Atkinson C** Role of oxidative phosphorylation in calcium homeostasis and changes in perfusion pressure following stimulation with noradrenaline in the rat tail artery

586P **Niederhoffer N, Amin F, Tatchum Talom R, Makki T & Atkinson J** Chronic angiotensin-converting enzyme inhibition counteracts the structural and functional consequences of arterial calcium overload

587P **Boutinet S, Giummelly P, Atkinson J & Capdeville-Atkinson C** Simultaneous measurement of intracellular calcium levels and the damping capacity of the rat common carotid artery in a new *in vitro* pulsatile system

588P **Mancuso F, Perretti M & Flower RJ** Investigation of dexamethasone effects on leucocyte behaviour using the hamster cheek pouch technique

589P **Whelan CJ, Hughes SC & Wren GPA** Differential sensitivity of PAF-induced eosinophilia and LPS-induced neutrophilia to dexamethasone in guinea-pig lung

590P **Holbrook M, Hughes P, Kingaby R, Hughes B & Higgs G** Dexamethasone inhibits allergen-induced eosinophil infiltration and activation in the guinea-pig lung

591P **English AF, Neate MS, Quint DJ & Sareen M** Some biological activities of a new corticosteroid, fluticasone propionate

592P **Cortijo J, Ortiz JL, Vallés JM & Morcillo EJ** Inhaled rolipram and zaprinast inhibit airway microvascular leakage produced by inhaled platelet-activating factor in the guinea-pig

593P **Clayton RA, Nally JE, Thomson NC & McGrath JC** The effect of oxygen tension on responses evoked by methacholine and bronchodilators in isolated bovine bronchial rings

594P **Ellis KE, Mistry R, Boyle JP & Challiss RAJ** Effect of charybdotoxin on inhibition of histamine-stimulated inositol phospholipid hydrolysis by isoprenaline, salbutamol and salmeterol in bovine tracheal smooth muscle

595P **Coleman RA, Nials AT & Johnson M** Potency, selectivity and duration of action of aralkyloxyalkyl analogues of β-adrenoceptor blocking drugs: parallels with salmeterol

596P **Hassan NAGM & Foster RW** Development of a method to assay salbutamol delivered from commercial formulations by its bronchodilator effects in normal human subjects

597P **McKean J & MacDonald A** Comparison of the effects of isoprenaline and BRL 37344 in tissues of the rat alimentary tract

598P **Morrison P, MacLean CM & Edwardson JM** Involvement of heterotrimeric G-proteins in the control of exocytotic membrane fusion in the exocrine pancreas

599P **Shih M-F & Taberner PV** Activation of adipocyte hormone-sensitive lipase in mice by β_3 adrenoceptor agonists

600P **Shad F, Hamilton CA & Reid JL** Oxidation of low-density lipoproteins in the presence of bovine aortic endothelial cells

601P **Wu XC, Richards NT & Johns E** Cytochrome P450-dependent monooxygenase: a major role in the vasoconstrictor responses of rat mesenteric arteries?

602P **Fishwick J, McLean WG, Edwards G & Ward SA** The toxicity of artemisinin, its derivatives and metabolites in Nb2a neuroblastoma and C6 glioma cells

603P **Smart D, Smith G & Lambert DG** Is Ca^{2+} channel opening responsible for the stimulation of $Ins(1,4,5)P_3$ formation by μ-opioids in SH-SY5Y cells?

604P **Houston AJ, Ebenezer IS & Wong CLJ** The effect of the opioid antagonist, naloxone, on baclofen-induced antinociception in male and female mice

605P **Davis AJ & Perkins MN** The involvement of bradykinin B_1 and B_2 receptor mechanisms in cytokine-induced mechanical hyperalgesia in the rat

606P **Davey PT, Hamilton TC & Sanger GJ** Divergent effects of olvanil in two acute models of nociception in the mouse

607P **Elliott P & Wallis DI** The actions of tachykinin agonists on single motoneurones in the neonatal rat spinal cord preparation

608P **Chizh BA, Cumberbatch MJ, Birch PJ & Headley PM** Neurokinins released tonically in the spinal cord of anaesthetized rats modulate excitatory amino acid efficacy

609P **Shepheard S, Williamson D, Cook D, Williams J, Sirinathsinghji D, Heavens R, Hill R & Hargreaves R** The NK_1 receptor antagonist CP-99,994 blocks dural extravasation and c-fos expression produced by electrical stimulation of the trigeminal ganglion in rats

610P **Mead AJ & Little HJ** Paired-pulse potentiation during ethanol withdrawal: changes in $GABA_B$ transmission?

611P **Richards DA, Lemos T, Whitton PS & Bowery NG** Increased extracellular GABA and taurine in the ventrolateral thalamus of rats with non-convulsive epilepsy

612P **Akhondzadeh S & Stone TW** Enhancement by muscimol of adenosine effects on hippocampal population spikes

613P **Hosseinzadeh H & Stone TW** On the mechanism of theophylline suppression of antidromic population spikes in rat hippocampus

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British Journal of Pharmacology

Proceedings Supplement

Proceedings of the British Pharmacological Society Meeting

Charing Cross and Westminister
Medical School

in conjunction with
Astra Neuro Science Unit

5th–7th January 1994

The proceedings of the British Pharmacological Society are published as a supplement to the *British Journal of Pharmacology*.

The *British Journal of Pharmacology* is published monthly by the Scientific & Medical Division, Macmillan Press Ltd.

All business correspondence and reprint requests should be addressed to the Scientific & Medical Division, Macmillan Press Ltd., Houndmills, Basingstoke, Hampshire RG21 2XS, UK. Telephone: (0256) 29242; Fax: (0256) 810526.

Annual subscription prices for 1994 EC £540, elsewhere £595/US$950 (sterling rate is definitive). Orders must be accompanied by remittance. Cheques should be made payable to Macmillan Press, and sent to: Macmillan Press Ltd., Subscription Department, Brunel Road, Houndmills, Basingstoke, Hampshire RG21 2XS, UK.

Overseas subscribers may make payments into the UK Post Office Giro Account No. 5192455. Full details must accompany the payment.

British Journal of Pharmacology (ISSN 0007-1188) is published monthly by Macmillan Publishers Ltd, c/o Mercury Airfreight International Ltd, 2323 Randolph Avenue, Avenel, NJ 07001, USA. Subscription price is $950.00 per annum. Second class postage paid at Rahway NJ. *Postmaster*: send address corrections to Macmillan Publishers, c/o Mercury Airfreight International Ltd, 2323 Randolph Avenue, Avenel, New Jersey, NJ 07001, USA.

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ISSN 0007-1188
0007-1188/94 $9.00 + $0.00

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British Journal of Pharmacology

Proceedings Supplement

Proceedings of the British Pharmacological Society Meeting

Victoria University
of Manchester

13th–15th April 1994

The *British Journal of Pharmacology* is published by Macmillan Press Ltd.

Scope The *British Journal of Pharmacology* is published monthly. It welcomes contribution in all field of experimental pharmacology including neuroscience, biochemical, cellular and molecular pharmacology. The Board of Editors represents a wide range of expertise and ensures that well-presented work is published as promptly as possible, consistent with maintaining the overall qulaity of the journal

This journal is covered by Current Contents, Excerpta Medica, BIOSIS and Index Medicus.

Editorial Manuscripts (plus two copies) and all editorial correspondence should be sent to: The Editorial Office, British Journal of Pharmacology, St George's Hospital Medical School, Cranmere Terrace, London SW17 0RE, UK. Tel: +44 81 767 6765; Fax: +44 81 767 5645.

Advertisements Enquires concerning advertisements should be addressed to: Michael Rowley, Hasler House, High Street, Great Dunmow, Essex CM6 1AP. Tel: +44 371 874613; Fax: +44 371 872273.

Publisher All business correspondence, supplement enquiries and reprint requests should be addressed to British Journal of Pharmacology, Scientific and Medical Division, Macmillan Press Ltd, Houndmills, Basingstoke, Hampshire RG21 2XS, UK. Tel: +44 256 29242; Fax: +44 256 810526. Publisher: Marija Vukovojac. Editorial Assistant: Alice Ellingham. Production Controller: Kath Parr.

Subscription Subscription price per annum (one volume, twelve issues) £540, rest of world £714 (Airmail), £595 (Surface mail) or equivalent in any other currency. Orders must be accompanied by remittance. Cheques should be made payable to Macmillan Magazines and sent to: The Subscription Department, Macmillan Press Ltd, Houndmills, Basingstoke, Hampshire RG21 2XS, UK. Where appropriate, subscribers may make payments into UK Post Office Giro Account No. 519 2455. Full details must accompany the payment. Subscribers from EC territories should add sales tax at the local rate.

British Journal of Pharmacology (ISSN 0007–1188) is published monthly by Macmillan Press Ltd, c/o Mercury Airfreight International Ltd, 2323 Randolph Avenue, Avenel, NJ 07001, USA. Subscription price for institutions is $950 per annum. 2nd class postage is paid at Rahway NJ. Postmaster: send address corrections to Macmillan Press Ltd, c/o Mercury Airfreight International Ltd, 2323 Randolph Avenue, Avenel NJ 07001.

Reprints of any article in this journal are available from Scientific and Medical Division, Macmillan Press Ltd, Houndmills, Basingstoke, Hampshire RG21 2XS, UK. Tel: +44 256 29242; Fax: +44 256 810526.

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ISSN 0007–1188

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Official publication of the British Pharmacological Society.

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Victoria University of Manchester
13th–15th April, 1994

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