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1P THE MEASUREMENT OF CEREBRAL EXTRACELLULAR K^+ BY ION-SELECTIVE MICROELECTRODES *IN VIVO*: EFFECT OF HYPOXIA AND MODULATION BY K^+ CHANNEL BLOCKING DRUGS

T. S. C. Zetterström and D. G. Grahame-Smith, Oxford University SmithKline Beecham Centre for Applied Neuropsychobiology, University Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE.

Ischaemia or anoxia leads rapidly to central nervous system (CNS) neuronal dysfunction. An early stage in the cytotoxic cascade of events is a disturbance of the ionic distribution across the neuronal membrane. Previous studies have shown that extracellular K^+($[K^+]_e$) increases in the brain during hypoxia or anoxia (Hansen et al, 1982). It has been assumed that this rise in $[K^+]_e$ is due to failure of energy provision for the ATP-dependent Na^+,K^+-pump (Masahiro et al, 1987). More recently a role for K^+-channels in mediating K^+-efflux induced by hypoxia has been suggested in particular ATP-sensitive K^+-channels (Ben Ari et al., 1990). We have established an in vivo microelectrode technique for real time measurements of $[K^+]_e$ in rat brain and studied the effect of short periods of hypoxia on the early increase of $[K^+]_e$ in rat hippocampus.

$[K^+]_e$ was measured using valinomycin-based double barrel ion-selective microelectrodes (2-6μm), constructed as described previously (Vaughan-Jones and Kaila, 1986). The reference barrel of these electrodes was back-filled with 150 mM NaCl and the ion-selective barrel with 150 mM KCl. Electrode calibration was carried out in saline-based solutions containing 3, 6 or 30 mM KCl. Only electrodes with calibration slopes greater than $55mV/\log_{10}$ of $[K^+]$ were used. Male Sprague-Dawley rats (250-300g) were anaesthetised with chloral hydrate 500 mg/kg i.p. and mounted in a stereotaxic frame. Gas mixtures were administered to rats by placing their snouts in a sealed piece of polyethylene tubing connected to gas cylinders. The body temperature of the rats were kept constant by using a thermostatic heating pad. Microelectrodes were implanted in dorsal hippocampus. The effect of transient hypoxia was studied by changing the control gas mixture (20% O_2 and 80% N_2) to 100% N_2 for 30s. Arterial blood gases were measured in femoral arterial blood samples using a blood gas analyzer. Statistical analysis were made using Students paired t-test.

Resting $[K^+]_e$ in these rats was estimated to be 3.4 ± 0.09mM, n=15 rats. Inhalation of 100% N_2 caused a highly reproducible rise in $[K^+]_e$, within 5-10s. After 30s inhalation of 100% N_2 the maximum rise of $[K^+]_e$ was 5-6mV (0.5-1mM). There was a rapid fall in $[K^+]_e$ within 5-10s of reintroducing control gas mixture which reached control levels within 20-30s. Levels of arterial pO_2 in control rats (15 ± 0.65kPa, n=3 rats) were reduced to 3.3 ± 0.85kPa, n=3rats during 30s of 100% N_2 inhalation. Pretreatment with quinine 50 mg/kg s.c. or 4-aminopyridine (4-AP) 1mg/kg s.c. 30min before the hypoxic challenge attenuated the increase in $[K^+]_e$ induced by 100 % N_2 inhalation for 30s by 35 ± 6.7 % (n=5rats, Mn±s.e.m, $p<0.05$) and by 70 ± 5.4% (n=4 rats, $p<0.05$) respectively compared to pre-drug measurements. In contrast gliquidone, 40μg in 10μl (1μl/min) i.c.v. administered 10 min before the hypoxic challenge had no effect.

In summary, we have established a method which is very sensitive and reproducible for the measurement of changes in brain $[K^+]_e$ in whole animals during transient hypoxia. This is a very early change in the cascade of events by which hypoxia causes neuronal damage. The demonstrated increase of hippocampal $[K^+]_e$ is attenuated by quinine and 4-AP. The lack of effect of gliquidone suggests that an ATP-sensitive K^+-channel may not be involved at this stage.

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2P CHLORMETHIAZOLE DOES NOT PROTECT AGAINST MPP+-INDUCED NIGRAL TOXICITY IN RATS

S. Rose, P. Jenner and A.R.Green[1]. Parkinson's Disease Society Experimental Research Laboratories, Pharmacology Group, Biomedical Sciences Division, King's College, Manresa Rd, London, U.K. [1]Astra Neuroscience Research Unit, 1, Wakefield Street, London, U.K.

Dizocilpine protects against methamphetamine (METH)-induced destruction of the nigrostriatal dopamine pathway (Sonsalla *et al*, 1989), and may prevent damage by 1-methyl-4-phenyl pyridinium ion (MPP^+) in rats (Turski *et al*, 1991). Chlormethiazole (CMZ) also protects against METH-induced destruction of the nigrostriatal dopamine neurones (Green *et al*, 1992). However, it is not known whether it protects against MPP^+-induced nigral damage. We report the effect of CMZ on damage to the nigrostriatal dopamine pathway induced by a focal injection of MPP^+ into the substantia nigra pars compacta (SNpc) of the rat.

Male Wistar rats (180-200g) were treated with CMZ (50mg/kg i.p.) or vehicle (0.9% saline) twice with 1h between treatments. Thirty minutes after the first injection of CMZ, lesions of the left SNpc were produced by the stereotaxic injection of MPP^+ (4μg in 1μl) using standard techniques under halothane anaesthesia. Sham lesions were made by injection of 0.9% saline (1μl) into the right SNpc. After 24h recovery, the animals were anaesthetised with pentobarbitone (60mg/kg i.p.) and transcardially perfused with ice cold 0.1M phosphate buffered saline and the brain removed. The position of the lesion was verified histologically using cresyl violet. Damage to the nigrostriatal pathway was determined by tyrosine hydroxylase (TH) immunostaining, [^{3}H]mazindol autoradiography and HPLC determination of dopamine, DOPAC and HVA.

There was 82% loss of TH-positive cells following MPP^+ administration into the left SNpc compared to the sham-lesioned side in control rats (Figure). CMZ did not alter the number or morphology of the TH-positive cells in either SNpc. [^{3}H]Mazindol binding was slightly reduced in the striatum ipsilateral to the lesion compared to the contralateral side in control rats. Again, CMZ did not alter [^{3}H]mazindol binding in either striatum. Striatal dopamine, DOPAC and HVA levels were unaltered both ipsilateral and contralateral to the lesion in CMZ-treated and control animals.

These data suggest that, unlike dizocilpine, CMZ is not neuroprotective against the dopaminergic neurotoxicity following a single injection of MPP^+ into the SNpc of rats.

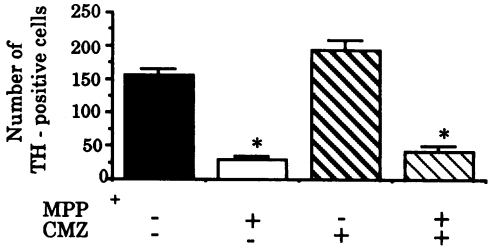

Figure The effect of CMZ on TH - positive cells in SNpc following MPP^+ administration to rats
* $p< 0.05$ compared to non-lesioned side (Students t-test)

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3P THE EFFECT OF CHRONIC VITAMIN E DEFICIENCY ON STRIATAL DOPAMINE LEVELS, REDUCED GLUTATHIONE AND TYROSINE HYDROXYLASE POSITIVE CELLS IN RAT SUBSTANTIA NIGRA

D. T. Dexter[1*], I. Nanayakkara[1], M. A. Goss-Sampson[2], D. P. R. Muller[2], C. D. Marsden[1] and P. Jenner[1]. [1]Parkinson's Disease Society Experimental Research Laboratories, Pharmacology Group, King's College, London, UK. [2]Division of Biochemistry, Institute of Child Health, London. UK.

^{18}F-DOPA PET scans in humans and striatal [^{3}H]-mazindol binding in rats, with a chronic vitamin E deficiency, have revealed evidence of nigrostriatal degeneration perhaps due to elevated oxidative stress (Dexter *et al*, 1993). We now report on the levels of dopamine and reduced glutathione in the striatum and the number of tyrosine hydroxylase (TH) immunoreactive cells in the substantia nigra (SN) of vitamin E deficient rats.

Male Wistar rats (n=12) were fed a vitamin E deficient diet (Dyets, USA) or the same diet supplemented with α-tocopherol acetate (40mg/kg, diet) for 52 weeks. Animals were killed by cardial perfusion with 5% dextrose saline whilst under anaesthesia, brains were removed and cut coronally at the level of the infundibular stem, the hind brain containing the SN which was fixed in 10% buffered formalin, whilst the striatia were dissected from the forebrain and rapidly frozen. Striatal dopamine was measured by HPLC with electrochemical detection, reduced glutathione (GSH) was measured by HPLC with UV detection after derivatisation, whilst TH immunohistochemistry was carried out on 20μm free floating sections using a polyclonal antibody. TH-positive cells were counted using a Zeiss microscope at six rostral caudal levels in the SN according to Carman *et al*, 1991.

In the striatum there was a small nonsignificant reduction in dopamine levels (by 17%) and in GSH (by 8%) in the vitamin E deficient rats compared to controls. However, there was a significant overall loss in TH positive cells in the SN of the rats fed on a vitamin E deficient diet for 52 weeks (Fig.1).

The results would indicate that the changes occurring in patients and rats with a chronic vitamin E deficiency is due to the loss of dopaminergic cell bodies in the SN.

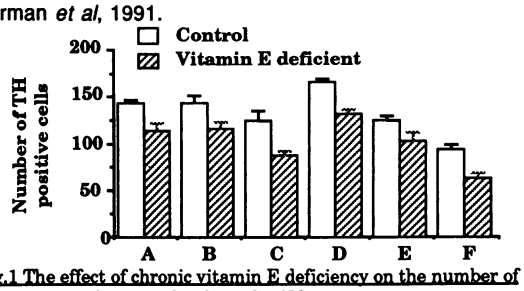

Fig.1 The effect of chronic vitamin E deficiency on the number of TH-positive cells at six levels in the SN.
Values represent mean± 1SEM. $p<0.05$ compared to control (Wilcoxon's test)

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4P A COMPARISON OF THE BENZAMIDE PHARMACOLOGY OF RAT AND HUMAN DOPAMINE D_3 RECEPTORS EXPRESSED IN CHINESE HAMSTER OVARY CELLS

C.Large, C.M.Stubbs, A.Mills*, E.Kawashima*, A.G.Hayes; Department of Neuropharmacology, Glaxo Group Research, Park Road, Ware, SG12 0DP. *Glaxo Institute for Molecular Biology, 14 Ch. des Aulx, 1228 Plan-Les-Ouates, Geneva, Switzerland.

The gene for a novel dopamine receptor, the D_3 receptor, has been cloned from both rats and humans (Sokoloff et al., 1990; Giros et al, 1990). The rat and human receptors are similar except for an extra 46 amino acid sequence in the putative third intracellular domain of the rat homologue. In this respect the rat and human D_3 receptors resemble two splice variants of the human dopamine D_2 receptor, $D_{2(long)}$ and $D_{2(short)}$ (Dal Toso et al., 1989). Recently, the long and short forms of the D_2 receptor have been distinguished by their affinities for substituted benzamide drugs (Castro and Strange, 1993). In view of these differences a comparison of the ligand binding properties of rat and human D_3 receptors has been carried out. The influence of sodium ions was also investigated, since the allosteric interaction of sodium with D_2-like receptors is known to affect binding of substituted benzamides (Neve, 1991).

Radioligand binding techniques, derived from the methods of Sokoloff et al. (1990), were used to investigate the pharmacology of rat and human dopamine D_3 receptors expressed in Chinese hamster ovary cells (cell lines were obtained from P.Sokoloff, INSERM, Paris). Binding of [^{3}H]-spiperone to rat and human D_3 receptors was characterised with saturation studies. Affinities of other compounds were measured by competition studies ([^{3}H]-spiperone concentration: 0.1nM). Values are the arithmetic mean ± s.e.m., n=3; slopes were close to unity. Data were analysed by a one way ANOVA followed by a post hoc Dunnett's test. No significant differences ($p>0.05$) were seen between the affinities of [^{3}H]-spiperone in the absence and presence of 120mM NaCl at rat (0.20±0.07nM; 0.29±0.07nM) and human (0.11±0.01nM; 0.23±0.03nM) D_3 receptors. The results of competition assays are shown in table 1.

Table 1

	Human D_3				Rat D_3			
	No NaCl		120mM NaCl		No NaCl		120mM NaCl	
n=3	pKi	s.e.m.	pKi	s.e.m	pKi	s.e.m	pKi	s.e.m
(-)Sulpiride	6.21	0.01	7.61*	0.21	6.53	0.13	7.85*	0.08
Clebopride	6.93	0.06	8.42*	0.19	7.07	0.09	8.50*	0.06
Raclopride	7.00	0.01	8.31*	0.22	7.07	0.09	8.10*	0.06
Haloperidol	8.20	0.05	8.03	0.18	7.97	0.03	7.83	0.15

The results indicate that rat and human D3 receptors show no significant difference ($p>0.05$) in their affinities for substituted benzamide and non-benzamide drugs. Furthermore, the removal of sodium ions has a similarly significant effect on the binding of substituted benzamides to both receptors (*$p<0.01$). The present results suggest that rat and human D3 receptors are very similar in respect of their antagonist binding characteristics for the compounds tested.

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5P PARTIAL CONVERSION OF D_1 DOPAMINE RECEPTORS FROM HIGH TO LOW AGONIST AFFINITY BY Gpp(NH)p, HEAT AND N-ETHYLMALEIMIDE

C.B. Farrell & K.M. O'Boyle, Department of Pharmacology, University College Dublin, Belfield, Dublin 4, Ireland

It has recently been reported that in the human brain two subtypes of D_1 dopamine (DA) receptors can be distinguished on the basis of differences in guanine nucleotide effects on agonist binding (De Keyser et al., 1989). In the amygdala, a complete conversion of high affinity states (R_H) to low affinity states (R_L) for DA was observed in the presence of guanine nucleotides whereas there was no guanine nucleotide-effect on R_H in globus pallidus. In caudate nucleus and nucleus accumbens, guanine nucleotides provoked only a partial conversion of R_H into R_L. These data suggest that despite similar pharmacological profiles, D_1 receptors in human brain differ in their coupling to G_S: guanine nucleotide-sensitive receptors, which are coupled to G_S, and guanine nucleotide-insensitive receptors, which are not. The present study sought to investigate the possible existence of such D_1 dopamine receptor subtypes in rat striatum using Gpp(NH)p, heat and N-ethylmaleimide (NEM) treatments and examining their effects on agonist interactions at D_1 receptors.
D_1 receptors in rat striatum were labelled with [^{3}H]SCH 23390 (0.5 nM) and binding was displaced with increasing concentrations of DA. The effect of Gpp(NH)p was examined by pretreating the membranes at room temperature for an hour with Gpp(NH)p prior to incubation with [^{3}H]SCH 23390. For experiments in which the effect of heat on ligand displacement was examined, membranes were heated at 60°C for 5 min after which the membranes were cooled on ice prior to the binding assays. For experiments in which the effect of NEM was examined, membranes were pretreated with 10 μM NEM at room temperature for 30 min prior to the assay. Data obtained was analysed using LIGAND (Munson & Rodbard, 1980) and the results are summarised in the Table.

	$\%R_H$	K_H, nM	K_L, nM	
Control	21 ± 4	87 ± 31	7091 ± 758	
Gpp(NH)p	10 ± 4	30 ± 14	10711 ± 937	means ± sem
Heat	10 ± 3	38 ± 18	8763 ± 824	n=5-9
NEM	12 ± 4	21 ± 14	6769 ± 605	

Displacement of [^{3}H]SCH 23390 with increasing concentrations of DA produced shallow displacement curves with 21% of [^{3}H]SCH 23390 displaying high affinity (R_H) for DA. Pretreatment with 100 μM Gpp(NH)p resulted in a partial conversion of R_H into R_L. Both heat and NEM pretreatments, previously shown to mimic the effects of guanine nucleotides (Kilpatrick et al., 1982) also only provoked a partial conversion of R_H into R_L. For all conditions, the apparent dissociation constants for the high- and low-affinity states (K_H and K_L) were similar. These data suggest that in rat striatum, as shown in human brain, two subpopulations of D_1 DA receptors may be distinguished on the basis of guanine nucleotide effects on agonist binding.

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6P A CLOZAPINE-INSENSITIVE RESPONSE TO THE DOPAMINE AGONIST, N,N-DIPROPYLAMINO-5,6-DIHYDROXYTETRALIN (dpADTN): REGIONAL LOCALISATION AND EFFECT OF PERTUSSIS TOXIN

D.R. Bull, A.G.Hayes and M.J. Sheehan[1], Departments of Neuropharmacology and [1]Gastrointestinal Pharmacology, Glaxo Group Research Ltd, Ware, Herts SG12 ODP, UK

Recently we presented evidence consistent with the existence of a novel type of dopamine autoreceptor on the cell bodies (substantia nigra) and terminals (striatum and nucleus accumbens) of dopaminergic neurones (Bull et al., 1993; Sheehan et al., 1993). The putative novel receptor was activated by dpADTN and was blocked by D_2 antagonists with lower potency than that required to block another D_2 agonist, quinpirole. Most notably, clozapine was very much weaker at blocking dpADTN than quinpirole. In the present study we have extended these findings to dopaminergic nerve terminals in the amygdala and investigated the effects of pertussis toxin in striatum.

Coronal slices, 350μm thick, containing either the anterior striatum or the central amygdaloid nucleus, were taken from the brain of male Wistar-derived rats (100 - 150g). Dopamine release was evoked by electrical pulses (one in striatum and two 10ms apart in amygdala), once every 2 min, and measured by fast cyclic voltammetry (Bull et al 1990). Drugs were added to the superfusate, which included dopamine D_1, 5-HT_1, 5-HT_2 and α-adrenoceptor antagonists. Pertussis toxin (8μg) was stereotaxically administered into the striatum 48hr before sacrifice.

Quinpirole and dpADTN (0.03-0.3μM) inhibited the evoked release of dopamine in both striatum (quinpirole EC_{50}=0.15±0.05μM, dpADTN EC_{50}=0.14±0.05μM) and amygdala (quinpirole EC_{50}=0.08±0.02μM, dpADTN EC_{50}=0.1±0.03μM). In the amygdala the quinpirole effect was blocked by sulpiride (pA_2=7.6, 95% confidence limits [6.9-7.9]), metoclopramide (7.5 [7.3-7.7]) and clozapine (6.3 [6.1-6.5]) (Bull et al., 1991) and the dpADTN effect was blocked with potency values not significantly different (7.4 [7.1-7.7], 7.6 [7.4-7.8], 6.9 [6.5-6.9] respectively). Antagonism of both agonists produced Schild slopes equal to 1. In contrast, in striatum the response to dpADTN was antagonised by sulpiride, metoclopramide and clozapine with potency values lower than those obtained for antagonism of quinpirole and Schild slopes were less than 1 (Sheehan et al. 1993).

In slices from animals pretreated with pertussis toxin the electrically evoked release of dopamine appeared to be both qualitatively and quantitatively the same. However, significant differences were seen in the degree to which quinpirole or dpADTN were able to inhibit this release. The inhibitory response to quinpirole was greatly reduced in striatal slices from complete (100%) inhibition in controls (n=3) to 40±8% (n=3) in slices from pertussis treated animals, whilst in the same slices the response to dpADTN was unaffected (n=3).

Thus, the putative novel dopamine autoreceptor which is activated by dpADTN and not blocked by clozapine appears to be present on the terminals of dopamine neurones in the striatum and nucleus accumbens but not in amygdala. Further evidence for its existence is given by the ability of pertussis toxin to block the response to quinpirole much more markedly than dpADTN. This may suggest that the putative novel receptor operates through the mediation of a different G-protein and/or second messenger.

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Bull, D.R.,et al. (1993) Br.J.Pharmacol., In press
Sheehan, M.J., et al. (1993) Br.J.Pharmacol., In press

7P EFFECTS OF TETANUS TOXIN ON BASAL AND K+-EVOKED EXTRACELLULAR CONCENTRATIONS OF 5-HT, DOPAMINE AND THEIR METABOLITES IN RAT HIPPOCAMPUS MEASURED BY *IN VIVO* MICRODIALYSIS

P. Britton, P.S. Whitton, L.J. Fowler and N.G. Bowery, Dept. Pharmacology, School of Pharmacy, 29/39 Brunswick Sq., London WC1.

Tetanus toxin (TeTx) produces alterations in hippocampal extracellular levels of 5-HT, dopamine and their metabolites up to 3 days after microinjection into the rat hippocampus (Whitton *et al.*, 1992). In particular, levels of 5-HT in toxin-injected hippocampi were reduced by up to 80% when compared to controls. TeTx is known to produce behavioural and neuropathological effects following injection into rat hippocampus (Bagetta *et al.*, 1990) but whilst neuronal degeneration is not evident until 7-10 days after treatment, the behavioural effects are manifested within 1-2 days. We have now determined, using *in vivo* microdialysis, the extracellular levels of 5-HT, dopamine, 5-HIAA and DOPAC in rat hippocampus 7 days after a single injection of TeTx.

Male Wistar rats (270-300g) were anaesthetized with chloral hydrate (400mg/kg) and TeTx (1000 mouse minimum lethal doses) injected unilaterally into the ventral hippocampus. Concentric dialysis probes were implanted 6 days after the initial toxin microinjection and dialysis was commenced 18-24h later. 30 min dialysis samples were collected from each hippocampi in freely moving animals throughout the duration of the experiment, following a 90 min stabilization period. After 2h the perfusion medium was switched to one containing 100mM K^+ for a 30 min period. The original medium was returned after this period and 3 more 30 min dialysis samples collected from each hippocampi. Both perfusion media contained 1μM citalopram. 7 days after injection of TeTx, mean levels of 5-HT and dopamine in toxin-treated hippocampi were significantly reduced compared to the control side (Table). No significant changes in extracellular levels of the metabolites 5-HIAA, DOPAC and HVA were detected in the toxin-treated side when compared to the control contralateral side. K^+ evoked the release of 5-HT and this was also obtained in toxin-treated hippocampus although the level was lower than in controls (Table).

DAY 7	HIPPOCAMPAL CONCENTRATION							
	5-HT (fmoles/10μl)		DA (fmoles/10μl)		5HIAA (pmoles/10μl)		DOPAC (fmoles/10μl)	
	Basal	*K^+ evoked*	*Basal*	*K^+ evoked*	*Basal*	*K^+ evoked*	*Basal*	*K^+ evoked*
Vehicle-treated	212 ± 20.3	707 ± 134.5*	49.7 ± 8.7	64.4 ± 13.7	32.2 ± 4.6	24.7 ± 1.9*	406 ± 118	409 ± 106
Toxin-treated	88.7 ± 16.7*	250 ± 73.6*	23.1 ± 3.9*	40.3 ± 8.8	25.1 ± 1.6	17.2 ± 4.4	181 ± 50	291 ± 130.7

*$p < 0.05$ using paired Student's "t" test n = 5 rats

In conclusion, 7 days after injection of TeTx there was a significant reduction in extracellular 5-HT and dopamine at the site. These levels corresponded to 41.8% and 46.5% of control basal levels respectively. The possibility that the observed changes may be produced in response to a primary alteration in another neurotransmitter cannot be excluded. PB is a SERC CASE student with Smithkline Beecham.

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8P 5-HT_{1A} RECEPTOR-ACTIVATION INCREASES cAMP EFFLUX IN THE RAT HIPPOCAMPUS *IN VIVO*

A.K. Cadogan, D.A. Kendall and C.A. Marsden. Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, U.K.

Cyclic AMP functions as an intracellular second messenger by activating cAMP-dependent protein kinases in response to activation of a number of neurotransmitter receptors. There is also evidence that there is an egress of cAMP out of a number of cells into the extracellular space (Barber & Butcher, 1983). Recently, *in vivo* studies using the technique of microdialysis have shown increases in extracellular cAMP in rat hippocampus following activation with serotonergic agonists (Sibjesma *et al.*, 1991). In the present study microdialysis was used to monitor extracellular changes in the cAMP concentration in the hippocampus of the freely-moving rat in response to a 5-HT_{1A} receptor agonist, 8-hydroxy-2-(dipropylamino)tetralin (8-OH-DPAT) and to examine the effects of a selective 5-HT_{1A} antagonist, WAY 100135 [N-tert-butyl 3- 4-(2-methoxyphenyl)piperazin-1-yl-2-phenylpropamide dihydrochloride].

Male, Lister hooded rats (270-300g) were implanted with microdialysis probes in the ventral hippocampus under halothane anaesthesia, as described by Wright *et al.* (1992). The probes were perfused with artificial cerebrospinal fluid at a constant flow rate of 1μl/min and 30 min samples were collected after a 3 h stabilisation period. Three control samples were taken followed by subcutaneous administration of saline (1ml/kg) or 8-OH-DPAT (0.3mg/kg) and samples collected for a further 3 h. For 5-HT_{1A} receptor antagonist evaluation, WAY 100135 (5mg/kg, s.c.) was administered 30 min prior to 8-OH-DPAT/saline. Forskolin (100μM) was perfused through the probe for 30 min after the control samples had been collected. cAMP levels in the perfusate were measured using a radioreceptor assay with [^{3}H]cAMP as described by Alexander *et al.* (1992).

The basal level of hippocampal extracellular cAMP was 2.3±0.2 pmol/ml (n=6) after the 3h stabilisation period. Perfusion of forskolin through the probe for 30 min significantly increased cAMP efflux (451±112%, $p< 0.05$, n=6) above that of basal. 8-OH-DPAT (0.3mg/kg, s.c.) caused a significant increase of 119±14% ($p<0.01$, n=6) in cAMP efflux, whereas a similar volume of saline had no effect. Desensitization of the 8-OH-DPAT-induced increase in cAMP efflux was observed following a second administration of 8-OH-DPAT 4h later. Administration of 8-OH-DPAT did not alter the forskolin-stimulated efflux of cAMP. Pretreatment with WAY 100135 prevented the 8-OH-DPAT-induced increase in cAMP efflux.

The data indicate that the 8-OH-DPAT-induced increase in cAMP efflux, seen in the rat hippocampus, is mediated by a 5-HT_{1A} receptor.

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9P GR127935: A POTENT ORALLY ACTIVE 5-HT_{1D} RECEPTOR ANTAGONIST

M. Skingle, D.I.C. Scopes[1], W. Feniuk, H.E. Connor, M.C. Carter[1], J.W. Clitherow[1] and M.B. Tyers. Departments of Neuropharmacology and [1]Medicinal Chemistry, Glaxo Group Research Ltd., Ware, Herts, SG12 0DP

Pharmacological investigation into the physiological role of 5-HT_{1D} receptors has been hampered by a lack of selective antagonists. We report on a novel, potent and orally active 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). The affinity of GR127935 at a range of receptor subtypes was assessed using radioligand binding methods (Hoyer, 1988; Hamblin et al, 1992). GR127935 potently inhibited binding to 5-HT_{1D} receptors and showed low-moderate affinity at 5-HT_{1A}, 5-HT_{1C} and 5-HT_2 receptors (Table 1). GR127935 had little or no affinity (pKi) at 5-HT_3 (5.2), 5-HT_4 (<5.0), 5-HT uptake (<5.0), α–adrenoceptor (<6.0), dopamine (<5.0), GABA (<5.0), histamine (<5.0) and muscarinic (<6.0) binding sites. Isolated rings of dog basilar artery (vascular 5-HT_1 receptor) were prepared (Connor et al., 1989). Sumatriptan-induced contractions were antagonised by low concentrations (1-10nM) of GR127935; with reduced maximum effect. The antagonism was reversible following extensive washing thus it is likely that the high lipophilicity (log P = 3.8) or slow dissociation rate of GR127935 results in an apparently insurmountable effect.

Table 1 Activity of GR127935 at 5-HT radioligand binding sites.

Receptor	pK_i	Tissue	[^{3}H] Ligand	Ligand [nM]
5-HT_{1A}	6.9	Rat hippocampus	8-OH-DPAT	0.5
5-HT_{1C}	6.4	Pig cortex	Mesulergine	1
5-HT_{1D}	8.5	Guinea-pig striatum	5-HT	1-2
5-$HT_{1D\beta}$	9.9	HeLa cell line	5-HT	0.5
5-HT_2	6.6	Rat cortex	Ketanserin	2

Unilateral infusion of 5-HT_1 receptor agonists into the substantia nigra in the guinea-pig elicits contralateral rotation via an action at 5-HT_{1D} receptors (Higgins et al., 1991). GR127935, 0.01-0.3mg/kg po, caused a dose-related inhibition of the contralateral turning elicited by a unilateral intranigral infusion of the 5-HT_1 receptor agonist GR56764 (1μg/2μl) (5-(3-methylamino-ethyl)-1H-indol-5-yl-1H-[1,2,4]triazol-3-yl) methanol). A dose of 0.3mgkg^{-1}p.o. inhibited the agonist response by greater than 75% for at least 8h. In marked contrast, GR127935, 0.1-10mgkg^{-1}s.c., failed to attenuate 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced (3mgkg^{-1} s.c.) wet-dog shakes in the guinea-pig; an effect which is potently inhibited by 5-HT_2 receptor antagonists (Skingle et al., 1991). These data suggest that GR127935 is a potent 5-HT_{1D} receptor antagonist which is likely to be useful in determining the role of 5-HT_{1D} receptors in the periphery and CNS.

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10P [^{125}I]-SB 207710, A POTENT, SELECTIVE RADIOLIGAND FOR 5-HT_4 RECEPTORS

A.M. Brown[1], T.J. Young[1], T.L. Patch[1], C.W. Cheung[2], A.J. Kaumann[3], L. Gaster[1] and F.D. King[1]. [1]Smithkline Beecham Pharmaceuticals, Coldharbour Road, Pinnacles, Harlow, Essex, CM19 5AD. [2]Smithkline Beecham Pharmaceuticals, The Frythe, Welwyn, Herts, AL6 9AR. [3]Clinical Pharmacology Unit, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ.

5-Hydroxytryptamine (5-HT_4) receptors have been identified by functional studies in brain, heart and gut (e.g. Bockaert et al., 1992) but detailed receptor analysis in terms of drug affinities has been hindered by the lack of a high specific activity radioligand binding assay. Recently, Grossman et al (1993) reported use of a ^{3}H-labelled 5-HT_4 receptor radioligand. We now describe an assay using [^{125}I]-SB 207710, ((1-butyl-4-piperidinylmethyl)-8-amino-7-iodo-1,4-benzodioxan-5-carboxylate), an analogue of the 5-HT_4 antagonist SB 204070 (Wardle et al., 1993). In guinea-pig distal colon, SB 207710 inhibited 5-HT_4 receptor-mediated contractions with an apparent pA_2 of 10.0. Its binding pK_i values were 6.3 or less at 5-HT_{1A} and 5-HT_3 receptors from rat cortex and at cloned human 5-$HT_{1D\alpha}$, 5-$HT_{1D\beta}$, 5-HT_{1E}, 5-HT_{2A} and 5-HT_{2C} receptors, indicating about 1000-fold selectivity for the 5-HT_4 receptor.

Piglet brain membranes were incubated (30 min, 37°C) in a buffer containing 50 mM Tris-HCl (pH 7.7 at 20°C), 4 mM $MgCl_2$, 100 μM pargyline and 0.2 mM ascorbic acid. Incubations were stopped by filtration over GF/B filters followed by washing with ice-cold buffer. Non-specific binding was defined using 5-HT (100 μM), or SB 205008 ((1-butyl-1-methyl-4-piperidinylmethyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate iodide) (10 μM), an analogue of SB 204070 with a pA_2 of 9.4 in guinea-pig distal colon.

Specific binding of [^{125}I]-SB 207710 (2-600 or 1300 pM) to piglet hippocampal membranes was saturable (K_D, 86±11 (4) pM; B_{max}, 16±3 (4) fmol.mg protein^{-1}; mean ± s.e.m (no of determinations)), reversible and had rapid kinetics (half times: 1-5 min for both association at 35 pM, and dissociation). In piglet caudate membranes, binding was of similar affinity (K_D: 37±11 (3) pM) and capacity (23±8 (3) fmol.mg protein^{-1}). In both tissues, 75-90% of binding was specific at 25-35 pM [^{125}I]-SB 207710 and was inhibited by a range of 5-HT_4 receptor agonists and antagonists (Table 1) with Hill slopes between 0.8 and 1.1. These affinity estimates are similar to functional data in piglet right atrium (Kaumann, 1990).

Table 1 Affinities of drugs at piglet hippocampal 5-HT_4 receptors

Drug	pK_i*
5-HT	6.6±0.1 (9)
5-Methoxytryptamine	6.2±0.1 (3)
Renzapride	6.2±0.1 (4)
Cisapride	7.2±0.1 (3)
Metoclopramide	5.5±0.1 (3)
ICS 205-930	6.4±0.1 (4)
SDZ 205-557	7.5±0.1 (3)
SB 207710	9.2±0.1 (4)
SB 205008	8.4±0.1 (3)

* -log(K_i), M; Mean ± sem (no. of determinations)

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11P VA-21B7: AN ATYPICAL 5-HYDROXYTRYPTAMINERGIC ANXIOLYTIC

J. Del Rio[1], I. Artaiz[1], A. Zazpe[1], G. Romero[1], A. Monge[2], J. Roca[3] & B. Lasheras[1], Departments of Pharmacology[1] and Organic Chemistry[2], Schools of Medicine and Pharmacy, University of Navarra, Pamplona, Spain; [3]Vita Research Department, Barcelona, Spain.

Several piperazinylindol derivatives were synthesized and studied as 5-HT_3 receptor antagonists. One of them, VA-21B7 (3-[2-(4^1-piperonylpiperazinyl)indoly]carboxaldeyde), was selected for further evaluation as an anxiolytic. *In vitro* VA-21B7 was only a moderately potent 5-HT_3 antagonist. The pA_2 against 2-methyl-5-HT in the isolated longitudinal muscle-myenteric plexus preparation of the guinea-pig ileum was 6.1 whereas those of the typical 5-HT_3 antagonists ondansetron and tropisetron were 6.9 and 8.3 respectively. The IC_{50} for displacement of [3H]-BRL-43694 binding to rat cortical homogenates was 0.2μM, thus being a weaker displacer, by one or two orders of magnitude, than ondansetron, granisetron or zacopride. It was also weaker than buspirone in the displacement of [^{3}H]8-OH-DPAT binding to cortical 5-HT_{1A} receptors, its IC_{50} being 8μM, almost two orders of magnitude higher than buspirone.

In spite of the low *in vitro* activity on the 5-HT receptors more usually associated with anxiety, VA-21B7 was a potent agent in different animal models used for the evaluation of 5-HT related anxiolytics, such as the two-compartment box in mice (light-dark exploration test), the social interaction test and the elevated plus-maze in rats (e.g. Rodgers and Cooper, 1991), and also in a classical conflict situation such as the punished-drinking (Vogel test) in rats. In the two-compartment box, VA-21B7 increased significantly, like ondansetron, the time spent in the clear compartment after i.p. or oral doses of 2-100 μg/kg. In rats, doses of 1-4mg/kg p.o. significantly increased, by approximately 30%, the time spent by pairs of animals in social interaction and also doubled the number of entries in the open arms of the elevated plus-maze. Interestingly, some 5-HT_3 antagonists (ondansetron, granisetron, tropisetron), as well as the 5-HT_{1A} agent buspirone, were more potent than VA-21B7 in the social interaction test but not in the elevated plus-maze. In this last test, ondansetron and buspirone were ineffective. In the Vogel test, the number of shocks accepted were also significantly increased, around 50%, after VA-21B7 (1-2mg/kg i.p. or 4-8mg/kg p.o.); ondansetron and tropisetron being also effective within a narrow dose range. Much higher doses of VA-21B7, up to 20mg/kg i.p., were necessary to decrease spontaneous motor activity in rodents and no overt neurotoxicity was apparent in doses up to 200mg/kg. After chronic administration in mice for 15 consecutive days (0.1mg/kg b.d.) no tolerance in the light/dark test or withdrawal syndrome was found. On the contrary, withdrawal from chronic diazepam (10mg/kg b.d.) produced a typical anxiogenic effect. VA-21B7, a potent anxiolytic in different animal models, is not obviously a GABA-related drug but the anxiolytic activity cannot be solely interpreted in terms of an antagonism at brain 5-HT_3 receptors.

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12P ETHOLOGICAL ANALYSIS FAILS TO REVEAL ANXIOLYTIC EFFECTS OF 5-HT_3 RECEPTOR ANTAGONISTS, ONDANSETRON AND WAY 100,289, IN THE MURINE PLUS-MAZE

R.J. Rodgers*, J.C. Cole and Tredwell J.M., Ethopharmacology Laboratory, Department of Psychology, University of Leeds, LS2 9JT, U.K.

5-HT_3 receptor antagonists have been reported to produce variable effects in animal models of anxiety (Shepherd et al., 1993) and, in particular, have generally failed to show a positive anxiolytic profile in the elevated plus-maze test. In the present study, we have assessed the effects of two 5-HT_3 antagonists, ondansetron (Glaxo) and WAY 100,289 (endo-N-[8-methyl-8-azabicyclo[3.2.1]octan-3-yl)aminocarbonyl]-2-cyclopropyl-methoxybenzamide; Wyeth; Bill et al., 1992) on the behaviour of male DBA/2 mice in the elevated plus-maze test using recently-developed ethological scoring methods (Rodgers & Cole, 1993). Naive mice were randomly allocated to treatment conditions (n=10) and administered (i.p.) saline, ondansetron (0.001-0.1 mg/kg) or WAY 100,289 (0.01-10.0 mg/kg) 45 min prior to testing. Tests were of 5 min duration, and sessions were recorded on videotape. Both traditional (% open arm entries, % open arm time) and novel (stretched attend postures, head-dipping, closed arm returns, entry latency, non-exploratory behaviour) behavioural measures were recorded.

ANOVA (df = 3,36) revealed that ondansetron was devoid of effects on total arm entries ($F = 0.9$), percent open entries ($F = 0.9$), percent open time ($F = 0.6$), head-dipping ($F = 0.7$), stretch attend postures ($F = 0.3$), closed arm returns ($F = 0.3$), entry latency ($F = 0.3$) and non-exploratory behaviour ($F = 0.01$). However, rearing (9.9 ± 0.6 vs control 5.3 ± 1.0, $P < 0.005$), percent closed arm time (54.4 ± 4.4 vs control 34.9 ± 7.0, $P < 0.025$) and percent protected head-dipping (head-dipping from secure areas of the maze; 85.6 ± 6.4 vs 64.1 ± 9.7, $P < 0.05$) were all increased at 0.001 mg/kg ondansetron, consistent with a mild anxiogenic action. WAY 100,289 was similarly without effect on most behavioural measures (df = 4,45): total entries ($F = 0.1$), rears ($F = 0.3$), percent open entries ($F = 1.39$), percent open arm time ($F = 0.5$), head-dipping ($F = 0.8$), stretch attend postures ($F = 0.8$), closed arm returns ($F = 1.2$) and non-exploratory behaviour ($F = 0.7$). Minor effects noted were an increase in centre platform time at 10.0 mg/kg (45.3 ± 3.0 vs control 35.6 ± 1.9, $P < 0.05$) and a reduction in entry latency at 1.0 mg/kg (10.2 ± 2.4 vs control 30.5 ± 5.1). Thus, despite the detailed analysis of behaviour currently employed, it would appear that the type of anxiety measured in the plus-maze is not sensitive to the putative anxiolytic effects of 5-HT_3 antagonists.

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13P PHARMACOLOGICAL EVALUATION OF THE ELEVATED "ZERO-MAZE" AS A MODEL OF ANXIETY IN RATS

J.K.Shepherd, S.S. Grewal, A. Fletcher, D. Bill & C.T. Dourish Wyeth Research (UK) Ltd., Maidenhead, Berks. SL6 0PH.

The elevated plus-maze is a valid model for the evaluation of putative anxiolytics in rats (e.g. Pellow et al., 1985). However, there are a number of potential problems associated with the traditional apparatus which can be eliminated by the present modified "zero-maze" design. Specifically, time spent on the central square (neither closed nor open) may compromise the interpretation of open/closed arm comparisons, while the 'box ends' of the closed arms prevent uninterrupted exploration. Thus, the "zero-maze" apparatus, comprising an elevated annular platform with two enclosed and two open quadrants, by nature of design, has no 'dead area' and allows continued forward exploration. In addition to physical modifications of the apparatus, a more detailed ethological analysis has been incorporated into the current procedure in order to expand the behavioural profile obtained with the test compound, and subsequently improve the validity of interpretation.

Male Sprague-Dawley rats (300-450g; n=10-12/group) were injected s.c. with either drug or vehicle (10% polyethylene glycol/saline) 30 min prior to being placed on a closed quadrant and a 5 min test period recorded on video for subsequent analysis. Behavioural measures comprised percentage time spent on the open quadrants, frequency of head dips over the edge of the platform, and frequency of stretch attend posture (SAP) from the closed to open quadrants. Drugs tested were diazepam (0.125-0.5 mg/kg), chlordiazepoxide (0.5-2.0 mg/kg), 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT; 0.001-0.1 mg/kg) and m-chlorophenylpiperazine (mCPP; 0.25-1.0 mg/kg). Diazepam (0.5 mg/kg) and chlordiazepoxide (2 mg/kg) significantly ($p<0.05$ or less; Kruskal-Wallis/Mann-Whitney) increased and mCPP (0.5-1.0 mg/kg) significantly decreased % time spent on the open quadrants, while 8-OH-DPAT had no significant effect. Similarly, diazepam (0.125-0.5 mg/kg) and chlordiazepoxide (0.5-2.0 mg/kg) significantly increased, and mCPP (1.0 mg/kg) significantly decreased, head dip frequencies. 8-OH-DPAT had no reliable effects on this measure. Finally, frequency of SAP was significantly decreased by diazepam (0.5 mg/kg), chlordiazepoxide (0.5-2.0 mg/kg) and 8-OH-DPAT (0.01 mg/kg), and significantly increased by mCPP (0.5-1.0 mg/kg).

The inclusion of more detailed behavioural measures in addition to % time spent on the open quadrants clearly enhances the sensitivity of the elevated "zero-maze" to the anxiolytic action of both benzodiazepines tested. However, the advantage of this form of analysis is best illustrated by present findings with the serotonergic compounds. The absence of any significant effect of 8-OH-DPAT on % time spent on the open quadrants is in keeping with the somewhat inconsistent results obtained with this compound using the elevated plus-maze (see Dourish, 1987). However, the observation that a low dose of 8-OH-DPAT decreased SAP in a similar manner to the benzodiazepines provides evidence of an anxiolytic effect. In addition, while interpretation of observed reductions in % time spent on the open and frequency of head dips following mCPP administration might have been confounded by the general decrease in activity previously reported with this compound (Kennett & Curzon, 1988), the observed increase in frequency of SAP strongly suggests an anxiogenic profile.

In conclusion, present data suggest that a combination of the novel "zero-maze" design and a detailed ethological analysis provides a sensitive model for the detection of anxiolytic/anxiogenic drug action.

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14P EFFECTS OF BENZODIAZEPINES ON CCK$_B$ RESPONSES RECORDED FROM RAT VENTROMEDIAL HYPOTHALAMUS *IN VITRO*

Boden,P. & Woodruff,G.N.
Parke-Davis Neuroscience Research Centre, Addenbrookes Hospital Site, Hills Road, Cambridge. CB2 2QB.

Bradwejn & De Montigny (1984) reported that activation of benzodiazepine receptors by benzodiazepine agonists blocked cholecystokinin(CCK)-induced excitation of rat cortical neurones *in vivo*. They proposed that this selective block of CCK responses by benzodiazepines acting *via* neuronal benzodiazepine receptors could account for the anxiolytic effects of these drugs. CCK$_B$ antagonists themselves have been shown to be anxiolytics (Hughes *et al.*, 1990). We have undertaken a study of the actions of the two benzodiazepines flurazepam and Ro15-1788 on CCK$_B$ responses recorded from neurones of rat hypothalamic (VMH) *in vitro*.

Recordings were made from a total of 26 VMH neurones (16 slices). Dose-response curves to the CCK$_B$-selective agonist pentagastrin were unaffected by either benzodiazepine at concentrations up to and including 10μM(n=5). In each experiment pentagastrin responses were attenuated by subsequent application of the CCK$_B$-selective antagonist CI-988. Application of flurazepam at 100μM did antagonize the pentagastrin response in an agonist-surmountable fashion, yielding a mean equilibrium constant for the antagonist (Ke) of 12.5μM (range 5.4-21.4μM, n=6). Extensive washing of the preparation for one hour with drug-free artificial cerebrospinal fluid restored the pentagastrin response which could then be antagonized by CI-988. Equilibrium constant values for the block produced by CI-988 were as expected (mean 4.3nM, range 2.0-8.2nM, n=4). The effects of flurazepam were specific for pentagastrin-induced increases in firing rate since carbachol responses were not altered in the presence of flurazepam (100μM, n=2). No effect on the pentagastrin dose-response curve was found when high (100μM) concentrations of the benzodiazepine antagonist Ro15-1788 were used but flurazepam (100μM) still blocked the pentagastrin response in the presence of Ro15-1788 with a Ke not different from that found in experiments performed in the absence of the benzodiazepine antagonist.

These data suggest that, in the rat hypothalamus, the CCK-antagonist effects of flurazepam are not due to an action at a benzodiazepine receptor *per se* but rather to a weak competitive block at the CCK$_B$ receptor.

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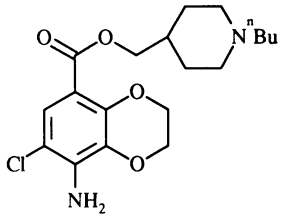
15P SB 204070: A HIGHLY POTENT AND SELECTIVE 5-HT$_4$ RECEPTOR ANTAGONIST

K.A.Wardle, E.S Ellis, L.M. Gaster, F.D.King and G.J.Sanger*. SmithKline Beecham Pharmaceuticals, Harlow, Essex CM19 5AD

By use of the guinea-pig distal colon longitudinal muscle myenteric plexus preparation (Wardle and Sanger, 1992) SB 204070 [(1-butyl-4-piperidinylmethyl)-8-amino-7-chloro-1,4-benzodioxan-5-carboxylate] has been identified as a highly potent and selective 5-HT$_4$ receptor antagonist.

In the presence of methiothepin (10^{-7}M) and granisetron (10^{-6}M), 5-HT (10^{-11}-10^{-8}M) evoked a monophasic concentration-dependent contraction with a pEC_{50} of 9.2 ± 0.08 (n = 38). SDZ 205 557 (Buchheit et al.,1991, 10^{-7}-10^{-6}M, n=6) produced a concentration-dependent shift of the curve to the right yielding a pA_2 of 7.8±0.1 and a slope of 1.06±0.09. SB 204070 (10^{-11}-10^{-8}M, n=6) in continuous contact with the tissue, produced a concentration-dependent rightward shift of the 5-HT curve at low concentrations (10^{-11}-10^{-10}M) and an additional reduction in the maximum at higher concentrations (10^{-10}M and above). This decrease in the maximum did not appear to be due to irreversible antagonism since the effects were reversed by washing. For example, the response to a pEC_{50} concentration of 5-HT was antagonised fully by SB 204070 (10^{-9}M) within 15 min. Following washout and re-dosing at 15 min intervals the response to 5-HT returned to control levels within 120 min. The apparent pA_2 value for the antagonist at10^{-11}M, 3x10^{-11}M and 10^{-10}M was 10.8 ± 0.1.



SB 204070

Table 1

Selectivity of SB 204070 (pK_i obtained using standard radioligands)

5HT$_{1A}$	<6
5HT$_{1C}$	7.0
5HT$_{1D}$	<5
5HT$_{1E}$	<5
5HT$_2$	<6
5HT$_3$	6.6
D$_2$	<5
D$_3$	<6
α_1, α_2	<6
β_1, β_2	<6

SB 204070 was selective when tested against other receptors (Table1) and did not affect DMPP-evoked contractions in the guinea-pig distal colon (at concentrations up to 10^{-6}M). These results show that in guinea-pig distal colon SB 204070 is a highly potent and selective 5HT$_4$ receptor antagonist.

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16P THE 5-HT$_4$ RECEPTOR ANTAGONIST SB 204070 INHIBITS THE CONTRACTILE RESPONSE TO 5-HT IN THE DOG HEIDENHAIN POUCH

Bingham, S*., King, B.F., Rushant, B., Smith, M.I. & Sanger G.J. SmithKline Beecham Pharmaceuticals, Harlow, Essex, CM19 5AD, UK.

In conscious dogs the Heidenhain pouch has been used to investigate the contractile effects of 5-HT in the gastrointestinal tract (Bermudez et al., 1990). This study ascertains which 5-HT receptors may be involved in mediating these effects.

Fasted dogs were used. The cephalic vein was cannulated acutely and 15 minutes later contractile responses to 5-HT (5 or 10 µg/kg iv) were recorded from the pouch via a pressure transducer, displayed on a chart recorder and stored on magnetic tape. 5-HT was administered every 30 minutes until two consistent consecutive doses were obtained. Fifteen minutes after the last dose of 5-HT, antagonists or saline were administered iv followed 15 minutes later by 5-HT. 5-HT was given at 30 minute intervals for 75 minutes after administration of antagonist. The recorded signal was integrated and the responses to 5-HT, obtained 75 minutes after each antagonist, expressed as a percentage of the mean control response for each dog which was taken as 100%. Results are shown in the table below.

Antagonist	Saline	Methysergide	Ketanserin	Methiothepin	Granisetron		Atropine	
µgkg^{-1}		100	100	100	10	100	30	100
(n)	(7)	(6)	(6)	(2)	(3)	(4)	(3)	(4)
% response	106.2 ± 13.2	126.9 ± 24.2	155 ± 55.3	113.1 ± 1.6	133.2 ± 23.8	99.2 ± 16.7	87.9 ± 22.8	25.4 ± 11.1

Antagonist				SB 204070			
µgkg^{-1}	0.1	0.3	0.5	1	3	30	100
(n)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
% response	71.5 ± 10.6	52.4 ± 11.1	41.2 ± 17.6	24.7 ± 20.7	-14.4 ± 21.9	18.6 ± 12.2	13.8 ± 16.1

Methysergide, ketanserin, methiothepin and granisetron had no consistent effect on contractile responses to 5-HT. Atropine had no effect at the lower dose but substantially reduced the response at the higher dose. The 5-HT$_4$ receptor antagonist SB 204070 (Wardle et al., this meeting) dose dependently reduced the response which was abolished between 0.5 and 1 µg/kg iv. An ID_{50} with 95% confidence limits was calculated as 0.55 (0.20,1.49) µg/kg.

These data indicate that in the conscious dog the contractile response to 5-HT in the Heidenhain pouch is not mediated by 5-HT$_1$, 5-HT$_2$ or 5-HT$_3$ receptors but does involve activation of muscarinic receptors and 5-HT$_4$ receptors. This model may be useful for investigating 5-HT$_4$ receptor function.

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Wardle, K.A., Ellis, E., Gaster, L, King, F. D. & Sanger, G.J. This meeting.

17P 5-HT_4 RECEPTOR ANTAGONISM BY SB 204070 INHIBITS 5-HYDROXYTRYPTOPHAN-EVOKED DEFAECATION IN MICE

Banner, S.E., Smith, M.I., Bywater, D. & Sanger, G.J., SmithKline Beecham Pharmaceuticals, Harlow, Essex, CM19 5AD, UK.

5-$HT_{1,2}$ and 3 receptors at best only have a minor role in 5-hydroxytryptophan (5-HTP) evoked defaecation in mice (Banner et al, 1993). Here we compare the effects of SB 204070 (Wardle et al, 1993) and other 5-HT_4 receptor antagonists on the 5-HTP induced facilitation of faecal pellet output in mice and thereby define a functional role for 5-HT_4 receptors.

Fed male CD1 mice (28-34g) were housed individually in mesh-bottomed perspex boxes mounted 5cm above the bench for 20min. After this acclimatisation period SDZ 205-557 (1-5000μgkg^{-1}), DAU 6285 (1-1000μgkg^{-1}), SB 204070 (0.003-1000μgkg^{-1}) or vehicle (saline) were dosed sc 5min prior to saline or 5-HTP (10$mgkg^{-1}$) sc. Faecal pellet output (FPO) numbers were recorded at 10min intervals and cumulative numbers calculated for each mouse.

In vehicle treated mice 5-HTP significantly increased FPO above saline levels with a maximum effect at 20min post-dose (336 $\pm$ 13%, $p<0.001$, n=130). At this time point each antagonist dose-dependently reduced 5-HTP evoked FPO, although SDZ 205-557 and DAU 6285 did not completely prevent the effect of 5-HTP. Thus, the metabolically unstable SDZ 205-557 (Ku et al, 1992) tended to reduce FPO at 100μgkg^{-1} (37 $\pm$ 20% inhibition, p=0.17, n= 10) and was maximally active at 5000μgkg^{-1} (45 $\pm$ 16%, p=0.08, n=10). DAU 6285 (1000μgkg^{-1}) also inhibited 5-HTP evoked FPO (54 $\pm$ 18%, p=0.04, n=10). SB 204070 was markedly more potent, tending to reduce FPO at doses as low as 0.3μgkg^{-1} (34 $\pm$ 15%, p= 0.08, n=15) and at 1000μgkg^{-1} maximally antagonised the effect of 5-HTP (83 $\pm$ 10%, p=0.03, n=15). None of the antagonists affected FPO in saline treated mice.

The 5-HTP increased defaecation in mice mimics the effects observed in volunteers (Davidson et al, 1957) and may model diarrhoea predominant irritable bowel syndrome (IBS). These results suggest that the pathophysiology of IBS could be related to a facilitatory role of 5-HT_4 receptors. However, compared with other 5-HT_4 receptor antagonists, only the use of the selective and highly potent antagonist, SB 204070 (Wardle et al, 1993), has allowed the response to 5-HTP to be characterised.

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18P THE HAEMODYNAMIC AND RENAL FUNCTIONAL RESPONSES TO THE 5-HT_{1A} RECEPTOR AGONIST FLESINOXAN IN THE ANAESTHETISED RAT

A.L. Chamienia and E.J. Johns, Department of Physiology, The Medical School, Birmingham B15 2TT, UK

It is well known that the sympathetic nervous system plays an important role in the control of the cardiovascular system. Furthermore renal sympathetic nerves exert a direct control on renin release, tubular sodium reabsorption and renal haemodynamics. In addition the increase in renal efferent and/or afferent nerve activity seems to exert an influence on the development of hypertension in animal models and in man. Evidence has accumulated to show that activation of 5-HT_{1A} receptors in the central nervous system decreases sympathetic outflow, including that to the kidney. The aim of the present study was to define the renal functional responses to i.v. administration of the novel 5-HT_{1A} agonist flesinoxan in normotensive Wistar rats.

Rats (mean body weight 300g) were initially anaesthetised with halothane and a cannula was inserted into the right femoral vein. Chloralose/urethane mixture was then given i.v. (initial dose 17.5mg α-chloralose, 0.3g urethane, followed by maintenance bolus injections of the same mixture as required) and an infusion of 3 ml h^{-1} saline was started. Cannulae were placed in the trachea and right femoral and carotid arteries for measurement of systemic and renal perfusion pressures, respectively. Both kidneys were approached retroperitoneally and their ureters cannulated. An electromagnetic flowmeter probe was placed around the left renal artery to allow measurement of renal blood flow. A loop of surgical thread was placed around the aorta between the renal arteries to facilitate manipulation of renal perfusion pressure. In denervation studies left (or both left and right) renal nerves were identified, isolated and cut. On completion of surgery, i.v. infusion of saline was replaced with one containing inulin (1.5 g 100 ml^{-1}) and animals were allowed to recover for 2 h. Data are expressed as means±SEM and statistical analysis was performed with repeated measures ANOVA.

In normotensive rats (n=8) flesinoxan at 30, 100, 300 and 1000 $\mu g\ kg^{-1}$ caused significant falls in blood pressure (BP) from 114±3 to 81±3 mmHg ($p<0.001$) and heart rate (HR) from 439±10 to 382±15 b min^{-1} ($p<0.001$) at the highest dose, but had no effect on renal blood flow or GFR. Despite the large falls in BP, urine flow (UV), absolute sodium excretion ($U_{Na}V$) and fractional sodium excretion (FE_{Na}) remained nearly unchanged. This was in striking contrast to the second group of animals (n=6), in which flesinoxan was not given, but renal perfusion pressure (RPP) was reduced to exactly the same levels as in the previous group by means of aortic constriction (AC). In this group UV, $U_{Na}V$ and FE_{Na} fell by 74-80% (all $p<0.001$) at the lowest pressure. In a further group of animals, subjected to left renal denervation (n=6) flesinoxan caused dose-dependant falls in BP and HR (both $p<0.001$); UV, $U_{Na}V$ and FE_{Na} all fell significantly by 67-71% (all $p<0.001$). In the subsequent three groups of rats, changes in RPP were prevented by applying AC and maintaining RPP at 80 mmHg. In control grooup of rats (n=7) renal excretion of water and sodium remained very stable as long as the pressure was controlled. In a second group (n=7), increasing doses of flesinoxan of 30, 100 and 300 $\mu g\ kg^{-1}$ caused dose-dependant significant increases in UV by 55.4% ($p<0.001$), $U_{Na}V$ by 186.5% ($p<0.005$) and FE_{Na} by 150% ($p<0.001$) at the highest dose. In the final group of experiments (n=7) flesinoxan was given to rats subjected to bilateral renal denervation as well as controlled perfusion pressure. The results obtained in this group were not different to those observed in the animals not receiving flesinoxan.

These results show that in a normotensive rat: 1. Novel centrally acting 5-HT_{1A} agonist flesinoxan reduces arterial pressure and heart rate in a dose-dependant manner. 2. Despite the fall in RPP the kidney function is well maintained in contrast to a situation when RPP is reduced by AC. The effect of flesinoxan on renal function appears to be via the renal nerves, since it is lost when the kidney is denervated. 3. When RPP is kept constant flesinoxan can induce a dose-dependant diuresis and natriuresis. This is prevented by renal denervation. 4. The above data suggest that, flesinoxan may be useful in the treatment of hypertension, specially in patients with impaired renal function.

19P THE ROLE OF α_1-ADRENOCEPTOR SUBTYPES IN THE REGULATION OF RENAL TUBULAR PROCESSES IN GENETIC HYPERTENSION

A.S. Munavvar* & E.J. Johns, Department of Physiology, Medical School, Birmingham, B15 2TT

The kidney is richly innervated by the sympathetic nervous system and both α_1 and α_2 adrenoceptors have been shown to be present in the renal cortex. Low frequency renal nerve stimulation has been demonstrated in the rat to produce antinatriuresis and antidiuresis which can be blocked by prazosin (Akpogomeh and Johns, 1990) thus suggesting that the α_1- adrenoceptor mediates the action of renal nerves on the epithelial cells. Recent studies showed that the α_{1a} subtype is predominantly involved in the regulation of renal haemodynamics in the stroke-prone spontaneously hypertensive rats (Munavvar and Johns, 1992). The aim of this study was to ascertain which subtypes of the α_1-adrenoceptors are involved in regulating the tubular reabsorptive processes in genetic hypertension. Male stroke-prone spontaneously hypertensive rats, 300-325g, were anaesthetised (sodium pentobarbital, 60 mg kg^{-1} i.p.). After tracheostomy, a carotid artery and jugular vein were cannulated for the measurement of blood pressure and infusion of saline. The left kidney was exposed via midline abdominal incision, 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 $\mu g\ kg^{-1}\ h^{-1}$ was infused. After the first set of five clearance collections, the second set was taken in the presence of chloroethyl-clonidine (CEC), an α_{1b}- alkylating agent or 5 Methylurapidil (5Me-U), an α_{1a}- antagonist both at concentrations of 10 $\mu g\ kg^{-1}\ h^{-1}$

Table 1

GROUP	VEHICLE		CEC		VEHICLE		5Me-U	
	CONTROL	EXPT	CONTROL	EXPT	CONTROL	EXPT	CONTROL	EXPT
RBF(ml kg^{-1} min^{-1})	13.0±0.7	9.3±0.8*	14.2±0.8	8.9±0.8*	14.3±0.8	10.7±0.8*	13.3±0.6	11.5±0.5
GFR (ml kg^{-1} min^{-1})	4.7±0.5	4.5±0.7	4.2±0.4	3.8±0.2	4.1±0.3	3.2±0.5	3.5±0.3	3.7±0.3
UV ($\mu l\ kg^{-1}\ min^{-1}$)	48.1±11.0	27.1±5.8*	51.0±11.3	17.1±5.4*	51.7±2.9	23.8±2.6*	53.8±8.1	59.0±8.5
$U_{Na}V$ (umol kg^{-1} min^{-1})	10.1±2.9	5.2±1.2*	10.7±3.1	2.2±0.7*	10.6±1.0	3.9±0.7*	11.9±2.3	13.7±2.6
FE_{Na} (%)	1.5±0.4	1.3±0.4	1.8±0.6	1.8±1.0	1.9±0.2	1.1±0.2	2.3±0.3	2.5±0.4
MAP (mmHg)	155±3	168±4*	138±2	167±4*	155±5	172±6*	150±2	158±3
RPP (mmHg)	151±3	151±3	135±4	134±4	156±4	158±4	150±2	152±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. *- $p<0.05$ *ys.* control, (ANOVA), n=6.

The results obtained in this study indicated that the regulation of renal tubular processes in the stroke-prone spontaneously hypertensive rat are mediated by the α_{1a}- adrenoceptors, as seen by their sensitivity to 5Me-U.

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20P AN INVESTIGATION OF THE RECEPTORS MEDIATING THE RESPONSES OF THE HUMAN SAPHENOUS VEIN TO PHENYLEPHRINE

R. Chess-Williams[1], B.M. Fabri[2], M.A. Fox[2], & A.H. Jackson[3]. Department of Biomedical Science, University of Sheffield[1], Cardiothoracic Centre, Liverpool[2] , and Department of Pharmacology and Therapeutics, University of Liverpool[3].

The human saphenous vein possesses a mixed population of postsynaptic α_1- and α_2-adrenoceptors (Roberts et al., 1992) and both α-adrenoceptor subtypes appear to contribute to the contractile responses to noradrenaline in this tissue. The present study examines the responses of the human saphenous vein to the relatively selective α_1-adrenoceptor agonist, phenylephrine.

Samples of human saphenous vein were obtained from patients undergoing coronary artery bypass surgery. The endothelium was removed and tissues set up in Krebs solution for the measurement of circular muscle contraction. Responses to phenylephrine were obtained in the absence and presence of prazosin (10nM-1μM) and WB4101 (10-100nM). The effects of preincubation with choroethylclonidine (25μM for 40min) on responses to phenylephrine were also studied. All experiments were performed in the presence of cocaine (10μM), corticosterone (10μM) and propranolol (1μM).

Increasing concentrations of prazosin or WB4101 did not result in concentration-related shifts of phenylephrine concentration-response curves. Experiments were therefore repeated in the presence of idazoxan (500nM). In these experiments, the Schild plot for WB4101 gave a straight line but the slope of the plot was significantly less than unity (slope = 0.33 $\pm$ 0.03, $P<0.05$) and the apparent pK_B value calculated from individual shifts was 7.8 $\pm$ 0.1 (n = 26). The Schild plot for prazosin appeared to consist of two components with intercepts of 9.0 (slope = 0.5) and 7.5 (slope = 0.8). Using 10nM and 30nM prazosin, an apparent pK_B of 8.4 $\pm$ 0.1 (n = 9) was obtained whilst 300nM and 1μM prazosin yielded an apparent pK_B of 7.2 $\pm$ 0.2 (n = 11). Idazoxan (500nM-1.5μM) antagonised responses to phenylephrine with an apparent pK_B of 7.2 $\pm$ 0.5. Incubating tissues with chloroethylclonidine in the absence of idazoxan increased phenylephrine EC_{50} values from 0.9(0.1-11.8) to 3.2(0.03-289)μM ($P<0.05$) but in the presence of idazoxan (500nM), chloroethylclonidine did not affect responses to phenylephrine.

These results suggest that the contractile responses of the human saphenous vein to phenylephrine are mediated by both α_1- and α_2-adrenoceptors.

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21P α_2 ADRENOCEPTOR-MEDIATED INHIBITION OF FORSKOLIN-STIMULATED cAMP ACCUMULATION IN THE PORCINE PALMAR LATERAL VEIN

I.K. Wright, D.A. Kendall and V.G. Wilson. Dept. of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham. NG7 2UH.

We have previously demonstrated the existence of α_2-adrenoceptors on porcine thoracic aorta and splenic arteries (Wright et al., 1993) and that α_2-adrenoceptor stimulation leads to a contractile response in the thoracic aorta (Blaylock and Wilson, 1993). Further radioligand and *in vitro* contractile studies have demonstrated the presence of functional α_2-adrenoceptors on other porcine vascular tissues including the palmar lateral vein (unpublished observations). It is well established that α_2-adrenoceptors are negatively linked to adenylyl cyclase and it is possible that a reduction in cyclic AMP (cAMP) might be the mechanism through which α_2-adrenoceptors produce a contractile response. The aim of this study was to determine whether α_2-adrenoceptor stimulation could inhibit forskolin stimulated cAMP formation in the porcine 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-adenine (specific activity = 23Ci/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, and agonists (noradrenaline (NA) or the selective α_2-adrenoceptor agonist UK14304 (10^{-9} - 10^{-4}M)) added for a period of 10 min prior to the addition of forskolin ($3x10^{-5}$M). Antagonists (rauwolscine or prazosin) were added 10 min before the addition of NA or UK14304. 5 min after the addition of forskolin the incubation was stopped by the addition of 200μl 1M HCl. ^{3}H-cAMP was separated from ^{3}H-adenine and other ^{3}H-products using sequential Dowex/alumina chromatography (Salomon et al., 1974). Radioactivity was determined using liquid scintillation counting at an efficiency of approximately 30%.

Forskolin ($3x10^{-5}$M) increased the levels of ^{3}H-cAMP to 785 ± 32% (n=12) above basal. This effect was inhibited in a concentration-dependent manner with either NA (pKi = 7.61 ± 0.37, n=3) or UK14304 (pKi = 7.84 ± 0.41, n=4). Neither NA or UK14304 altered basal cAMP levels. The α_2-adrenoceptor antagonist rauwolscine produced a concentration-dependent reversal of the inhibitory effect of UK14304 (10^{-6}M) with a pKi = 8.35 ± 0.39 (n=3), but had no effect alone on either basal or forskolin stimulated cAMP formation. The α_1-adrenoceptor antagonist prazosin (10^{-5}M) and the ß-adrenoceptor antagonist propranolol (10^{-5}M) did not reverse UK14304 induced inhibition of forskolin stimulated cAMP formation and had no effects on basal or forskolin-stimulated cAMP levels.

The results demonstrate the existence of functional α_2-adrenoceptors in the porcine palmar lateral vein, stimulation of which results in the inhibition of forskolin stimulated cAMP formation. Further studies are required to determine whether inhibition of cAMP formation is required to produce the contractile responses observed in response to α_2-adrenoceptor stimulation *in vitro*.

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22P CHRONIC ETHANOL INCREASES BASAL, AGONIST- AND FORSKOLIN-STIMULATED CYCLIC AMP ACCUMULATION IN NG108-15 CELLS

E. Kelly & R.J. Williams, Department of Pharmacology, University of Bristol, Bristol BS8 1TD

Chronic ethanol has previously been reported to induce heterologous desensitization of receptor stimulated cyclic AMP accumulation in NG108-15 cells apparently resulting from a selective reduction in Gsα expression (Mochly-Rosen *et al.*, 1988). However we have recently shown that chronic incubation of NG108-15 cells with ethanol reduces the expression not only of Gsα but also Giα_2, whilst Goα expression is increased. Furthermore this treatment enhances basal and agonist stimulated cyclic AMP accumulation (Williams *et al.*, 1993). Here we further investigate the effects of chronic ethanol on cyclic AMP signal transduction in NG108-15 cells.

Preconfluent NG108-15 cells were incubated with varying concentrations of ethanol for 48h, washed, and cyclic AMP accumulation measured for an 8 min period in the presence of Ro20-1724 (100μM) using a protein binding assay (Williams *et al.*, 1993). Chronic ethanol treatment (200mM;48h) increased basal (control 18.6 +/- 1.6; chronic ethanol 32.1 +/- 4.5 pmoles cAMP/min/mg protein), 10μM iloprost-stimulated (control 17.6 +/- 7.9; chronic ethanol 48.7 +/- 4.7pmoles cAMP/min/mg protein) and 10μM forskolin-stimulated (control 36.3 +/- 15.9; chronic ethanol 215.7 +/- 43.0 pmoles cAMP/min/mg protein) cyclic AMP accumulation (all $p<0.05$, n=6-7). Concentration effect curves for iloprost indicated that chronic ethanol did not affect iloprost's potency (control EC_{50} = 58 +/- 23nM; EC_{50} following chronic ethanol = 28 +/- 14nM, n=3). Interestingly chronic ethanol did not affect basal (96.8 +/- 7.2% of control), 10μM iloprost- (89.0 +/- 7.4% of control), 10mM NaF- (92.0 +/- 6.5% of control), or 10μM forskolin-stimulated (96.0 +/- 3.2% of control) adenylyl cyclase activity in cell homogenates. Replacement of 200mM ethanol during the 8 min incubation following chronic ethanol and washing did not reverse the chronic ethanol induced increase in basal, iloprost- or forskolin-stimulated cyclic AMP accumulation in intact cells. We have previously reported (Williams *et al.*, 1993) that acute addition of adenosine deaminase to cells during the 8 min incubation abolishes the chronic ethanol induced increase in basal cyclic AMP accumulation, but does not affect the chronic ethanol induced increase in iloprost stimulation. Acute inclusion of adenosine deaminase (1 unit/ml) reduced the 10μM forskolin-stimulated cyclic AMP accumulation in intact cells (control cells 34.9 +/- 15.0% reduction and chronic ethanol treated cells 59.5 +/- 12.3% reduction, n=4). However, a significant component of the forskolin-stimulated response remained, which was greater in ethanol treated cells (363 +/- 56% of control, n=4).

Thus, the increase in basal cyclic AMP accumulation following chronic ethanol is totally adenosine dependent, that due to forskolin appears partially so and that due to iloprost is resistant to the removal of extracellular adenosine. We conclude that chronic ethanol, apart from altering the ability of NG108-15 cells to synthesize/release/take up adenosine, also alters the sensitivity of the cyclic AMP signal transduction system to agonist and forskolin stimulation. However an increase in membrane levels of adenylyl cyclase appears unlikely since the increased sensitivity is not observed in cell homogenates.

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23P EFFECTS OF GLIBENCLAMIDE AND CHARYBDOTOXIN ON RELAXATIONS TO THE POTASSIUM CHANNEL ACTIVATOR BRL-55834 IN GUINEA-PIG TRACHEA

Kim Lawson, Martine Barras, J. Michael Armstrong & Peter E. Hicks. Department of Pharmacology, Recherche Syntex France, 91310, Leuville-sur-Orge, FRANCE.

BRL-55834, which demonstrates airways selectivity *in vitro* relative to BRL-38227 (Taylor *et al.*, 1992), has been reported to activate both a small conductance (44 pS) ATP- and glibenclamide-sensitive K^+ channel and, at higher concentrations, a large conductance (243 pS) charybdotoxin-sensitive, Ca^{2+} activated K^+ channel in bovine trachealis smooth muscle cells (Ward *et al.*, 1992). We have therefore compared the effects of the antagonists, glibenclamide and charybdotoxin (CTX), on BRL-55834-induced relaxations of spontaneous tone in guinea-pig trachea (GPT).
Tracheal rings from male Dunkin-Hartley guinea-pigs were suspended in Krebs bicarbonate solution under a resting tension of 2 g. Cumulative concentration-response curves to BRL-55834 (0.01-30 μM) were constructed 30 min after exposure to vehicle or glibenclamide (0.1, 0.3, 1.0, 3.0 or 10 μM), CTX (0.1 μM), CTX (0.1 μM) plus glibenclamide (3.0 μM). Relaxant responses were determined as a % of a maximal relaxation to aminophylline (1 mM). pIC_{30} (concentration required to evoke a response equivalent to 30% of the aminophylline maximum) and E_{max} (maximum response) values are reported as mean ± s.e.means for n determinations.
BRL-55834 evoked relaxations of GPT (pIC_{30} 7.66 ± 0.07, E_{max} 76 ± 8%, n = 16) and the concentration-response curve was displaced to the right, with no change in the E_{max}, in a concentration-related parallel manner by glibenclamide (0.1 - 3.0 μM). Schild analysis gave a pA_2 value of 7.74 (n = 34) with a slope of 0.83. The higher concentration of glibenclamide (10 μM) failed to displace the BRL-55834 curve further than 3 μM glibenclamide (pIC_{30} values after 3 μM glibenclamide 5.81 ± 0.19, n = 11 and after 10 μM glibenclamide 6.30 ± 0.19, n = 7). The pK_B value for glibenclamide (10 μM) was 6.37 ± 0.17 (n = 7), which was significantly less than the pA_2 value obtained with glibenclamide (0.1 - 3.0 μM). CTX (0.1 μM) alone failed to modify the BRL-55834-induced relaxations of GPT (pIC_{30} control 7.55 ± 0.08, after CTX 7.35 ± 0.17, n = 6). BRL-55834 concentration-response curves in the presence of CTX (0.1 μM) plus glibenclamide (3 μM) were not different from those obtained after glibenclamide (3 μM) alone (pIC_{30} values control 7.67 ± 0.08, after glibenclamide 5.43 ± 0.20 and after CTX plus glibenclamide 5.38 ± 0.16, n = 4). E_{max} values of BRL-55834 in the presence of glibenclamide or CTX, alone or in combination were not different from control values.
In conclusion, BRL-55834 (up to 1 μM) evoked glibenclamide-sensitive relaxations of the spontaneous tone of GPT. At higher concentrations (≥1 μM), however, the responses to BRL-55834 involved a glibenclamide-resistant mechanism, which was not blocked by CTX at a concentration previously shown to inhibit salbutamol or sodium nitroprusside-induced relaxations of GPT (Jones *et al.*, 1990; Hamaguchi *et al.*, 1992). These findings demonstrate that two distinct mechanisms (one sensitive and the other resistant to glibenclamide), which do not involve activation of CTX-sensitive Ca^{2+} activated K^+ channels (Ward *et al.*, 1992), are involved in the relaxation responses to BRL-55834 in guinea-pig trachea.

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24P ACTIONS OF DOXAPRAM ON Ca^{2+}-ACTIVATED K^+ CHANNELS FROM ISOLATED TYPE I CAROTID BODY CELLS OF THE NEONATAL RAT

C.N. Wyatt & C. Peers, Department of Pharmacology, Leeds University, Leeds LS2 9JT.

Previous studies have shown that doxapram, a respiratory stimulant, inhibits whole-cell K^+ currents (both Ca^{2+}-dependent (IK_{Ca}) and Ca^{2+}-independent components) in isolated type I carotid body cells of the neonatal rat (Peers, 1991). Here, we report studies of the single channels which underlie the whole-cell IK_{Ca}. Type I cells were isolated as previously described (Wyatt & Peers, 1992), and recording conditions were essentially similar to those used for whole-cell studies (21-24°C, 'intracellular' [ATP] 2mM, pH_i 7.2, pH_o 7.4, buffered with HEPES).

Using symmetrical 120mM [K^+] solutions, channels were identified in excised, outside-out patches with a slope conductance of 187±4pS (mean±s.e.m., n=9). At 0mV, reducing the extracellular [K^+] to 5mM revealed channels of amplitude 5.50±0.18pA (mean±s.e.m., n=11 patches). All subsequent experiments were carried out under these conditions. Activity of these channels was greater when the pipette 'intracellular' free [Ca^{2+}] was raised from 10^{-7}M to 10^{-6}M (NP_o 0.0010±0.0002 (n=7) and 0.627±0.110 (n=19) respectively, $p<0.005$, unpaired t-test). At any given $[Ca^{2+}]_i$, channel activity increased with increasing membrane potential. NP_o in inside-out patches was 0.0026±0.0005 (n=5, $[Ca^{2+}]_i$ 10^{-7}M) and raising 'intracellular' [Ca^{2+}] to 10^{-6}M always increased channel activity (NP_o rose to 0.031±0.001 in the same 5 patches, $p<0.002$, paired t-test). This value for NP_o at 10^{-6}M [Ca^{2+}] was far lower than in outside-out patches, an observation for which we cannot account at present. Nevertheless, these data indicate that this channel is a Ca^{2+}-dependent K^+ channel, known to be present in whole-cell recordings (Peers, 1991).

Bath application of charybdotoxin always produced a rapid, powerful inhibition of these channels. In 5 outside-out patches NP_o fell from 0.76±0.21 to 0.06±0.02 when 100nM charybdotoxin was applied ($p<0.05$, paired t-test). This inhibition was only slowly reversible.

During bath application of doxapram (30μM to 300μM), open channel noise increased in a concentration-dependent manner and the mean unitary current amplitude was reduced. For example, in the presence of 300μM, unitary amplitude was reduced from 5.01±0.18pA to 3.81±0.22pA (n=9 patches, $p<0.0005$, paired t-test). These results indicate that doxapram can directly modulate Ca^{2+}-dependent K^+ channels in type I carotid body cells, and are consistent with doxapram causing a 'flickery' type block (see Davies et al., 1991).

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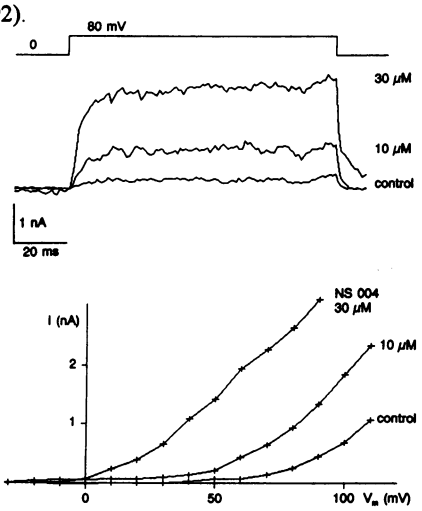
25P A NOVEL BENZIMIDAZOLONE, NS004, ACTIVATES LARGE-CONDUCTANCE Ca^{2+}-DEPENDENT K^+ CHANNELS IN AORTIC SMOOTH MUSCLE

S-P Olesen, F Wätjen & AG. Hayes*, NeuroSearch A/S, 26B Smedeland, DK-2600 Glostrup, Denmark and *Glaxo Group Research Ltd., Ware, Hertfordshire SG12 0DP, UK

Large-conductance Ca^{2+}-dependent K^+ channels, or BK channels, are present in most smooth muscle cells, and due to their large unit conductance may significantly modulate cell excitability when activated. The activation of BK channels is regulated by internal Ca^{2+}, membrane potential and/or phosphorylation; the known potassium channel openers do not influence this channel. We report here patch-clamp experiments on the activation of BK channels in aortic smooth muscle by a novel compound, NS004 (1-(2'-hydroxy-5'-chlorophenyl)-5-trifluoromethyl-2(3H) benzimidazolone) (Olesen & Wätjen 1992).

Smooth muscle cells were isolated and cultured from calf aorta and single BK channels were studied using the patch-clamp technique (inside-out and outside-out mode). The channels had a unit conductance of 278±5 pS (mean ± s.e.; symmetrical 146 mM K^+), were activated by internal Ca^{2+} (30-1000 nM), and were blocked by external charybdotoxin (20-100 nM) and TEA^+ (0.3-1 mM). NS004, administered to the bath, reversibly activated the channels after a delay of 1-3 min; (10 and 30 µM NS004 increased the open probability by 2.8±0.5 fold and 6.2±1.4 fold, respectively). NS004 increased the channel mean open time as well as the opening frequency, but did not induce new kinetic states. In whole-cell recordings the cells expressed a large, outward BK current at positive membrane potentials. NS004 significantly increased this current as shown in the figure. The effect of NS004 was to shift the activation curve towards negative membrane potentials; the average effects of 10 and 30 µM NS004 were leftward shifts of -10.3±5.6 mV and -64.0±4.0 mV, respectivley. TEA^+ (1 mM) blocked the NS004-induced current as well as the control current, whereas glibenclamide (1 µM) failed to influence these currents. In cultured cerebellar granule cells NS004 activated BK currents with a similar potency, without influencing Na currents or the delayed rectifier potassium current.



In conclusion, NS004 is an effective activator of BK currents in arterial smooth muscle cells, and this may be a novel pharmacological approach to smooth muscle relaxation.

Olesen, S-P & Watjen, F (1992) Benzimidazole derivatives, their preparation and use. European Patent Application no. EPA-477819.

26P DEQUALINIUM, A SELECTIVE BLOCKER OF THE K^+ CHANNEL UNDERLYING THE SLOW AFTER-HYPERPOLARIZATION IN RAT SYMPATHETIC NEURONES IN CULTURE

P.M. Dunn, Dept of Pharmacology, University College London, Gower Street, London WC1E 6BT.

Decamethylene bis 4-aminoquinaldinium (dequalinium) has been shown to block the apamin-sensitive small conductance, calcium activated potassium (SK) channel in guinea pig hepatocytes (Castle *et al.*, 1993). In rat sympathetic neurones, the action potential is followed by a long lasting after-hyperpolarization (AHP) which is due to the opening of calcium activated potassium channels and is composed of apamin-sensitive and -insensitive components (McAfee & Yarowski, 1979; Kawai & Watanabe, 1986). In addition, these cells also possess three types of voltage activated potassium channels which give rise to the membrane currents I_m, I_K, and I_A (Brown *et al.*, 1982). In this study, the ability of dequalinium to inhibit the slow component of the AHP has been compared with its ability to block the four other types of potassium current in these cells.

AHPs were recorded in cultured rat sympathetic neurones using conventional microelectrodes filled with 1M KCl. Potassium currents were recorded using the whole cell patch clamp technique.

Dequalinium produced a rapid and reversible inhibition of the slow phase of the AHP, leaving only the rapidly decaying apamin-insensitve component. This effect of dequalinium was concentration dependent, and least squares fitting of a simple mass action curve to the combined data from 23 cells gave an IC_{50} of 1.0 ± 0.1 µM (fit ± S.D.). When dequalinium (10µM) was applied in the presence of 20nM apamin to block almost all the SK channels, there was no reduction in the residual AHP. Under voltage clamp, dequalinium (1µM) produced 45 ± 2 % (mean ± s.e.mean; n=47) inhibition of the underlying membrane current I_{AHP}. In accutely dissociated neurones (in which I_{AHP} is absent) depolarization from a holding potential of -50mV to -10mV evoked a composite outward current made up of I_C and I_K, which was only inhibited by 2.6 ± 1.5% (n=4) by 10µM dequalinium. The A-current activated by returning to a holding potential of -50mV after a 500ms hyperpolarizing pulse to -100mV was reduced by 8 ± 5% (n=4) by 10µM dequalinium. However this concentration of dequalinium produced 18.3 ± 4.5 % (n=8) inhibition of the 'm'-current.

The potency and selectivity of dequalinium, together with its rapid action and reversibility, should make it a useful probe for the study of the SK channel.

Supported by the Wellcome Trust.

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27P THE EFFECT OF ADMINISTRATION OF NERVE GROWTH FACTOR ON CALCITONIN GENE-RELATED PEPTIDE (CGRP) EXPRESSION IN SCIATIC PRIMARY AFFERENTS OF DIABETIC RATS

L.T. Diemel, W.J. Brewster, P. Fernyhough and D.R. Tomlinson Department of Pharmacology, Queen Mary and Westfield College, Mile End Road, London E1 4NS, U.K.

Experimental diabetes in rats is associated with a reduction in the levels of sciatic nerve substance P and calcitonin gene-related peptide (CGRP) (Diemel *et al.*, 1992). Sensory neurone dysfunction is a hallmark of diabetic distal symmetrical polyneuropathy, both CGRP and SP are present in these neurones, and it has been suggested that impaired neurotrophic support of the sensory neurones in diabetes may be responsible for the reduction in sciatic nerve neuropeptides. Nerve growth factor (NGF) is a target-derived factor that has been shown to up-regulate CGRP and SP gene expression in dorsal root ganglia cell culture. This study was designed to determine whether the administration of NGF could oppose diabetes-induced deficits in peripheral nerve neuropeptide content. We also measured the CGRP mRNA content of the L_4 and L_5 dorsal root ganglia from the same animals to determine whether the diabetes induced peptide deficit is derived from a reduction in its mRNA and whether any effect of NGF was registered as altered CGRP mRNA levels. The mRNA was measured by Northern blot hybridisation with a cDNA, labelled with [^{32}P] by asymmetric polymerase chain reaction and expressed in densitometric units, relative to internal standard (a truncated 'sense' cRNA added at the start of RNA extraction). Duration of diabetes (induced with streptozotocin 50 mg/kg i.p.) was 6 weeks and diabetic rats were treated subcutaneously with vehicle (normal saline) or NGF (0.5 mg/rat s.c. 3 times per week) for the final three weeks of the study. Non-diabetic control rats also received the injection vehicle for an equivalent period. All data are mean ± S.D.; significant differences were derived by one-way ANOVA with Duncan's range tests applied where $F<0.05$. NGF treatment did not attenuate the metabolic severity of the diabetes, as judged by a lack of reversal of the hyperglycaemia or weight loss in the NGF-treated diabetics. Sciatic nerves of diabetic rats treated with vehicle had significantly reduced CGRP (10.00±3.62 [ng/mg nerve protein]) compared to controls (13.89±1.42; $p<0.05$). Diabetic rats treated with NGF had significantly higher levels of CGRP (18.75±3.62) than either control or diabetic vehicle-treated rats ($p<0.05$ for both). CGRP mRNA was significantly reduced in the L_4 and L_5 DRG of the vehicle-treated diabetic rats (2.73±0.62 *versus* controls 3.75±0.95; $p<0.05$). NGF treatment reversed this deficit (5.00±0.38; $p<0.05$ *versus* vehicle-treated diabetics). There were no changes in poly A^+ levels (measured on slot-blots by hybridization with a poly dT oligonucleotide probe) in the dorsal root ganglia. The increase in CGRP mRNA in the NGF treated diabetic rats was therefore a specific effect of the treatment. These data indicate that NGF either functionally antagonises or prevents a diabetes-induced deficit in the expression of CGRP at the mRNA level.

Diemel, L.T., Stevens, E.J., Willars, G.B. & Tomlinson, D.R. (1992) Neurosci. Lett. 137, 253-256.

28P NERVE ISCHAEMIA IN DIABETIC RATS: TIME-COURSE OF DEVELOPMENT AND EFFECTS OF INSULIN OR ALDOSE REDUCTASE INHIBITION

E.J. Stevens & D.R. Tomlinson, William Harvey Research Institute, Department of Pharmacology, Queen Mary and Westfield College, Mile End Road, London E1 4NS, UK.

Nerve disorders in experimental diabetes are associated with and may be caused by endoneurial ischaemia. We investigated diabetic nerve ischaemia via measurements of sciatic nerve laser Doppler flux in two studies. The first examined the time-course of development of a deficit in nerve laser Doppler flux in streptozotocin-diabetes, using untreated control and diabetic rats at timepoints over 8 weeks. The second study determined the effect on sciatic nerve Doppler flux of either 4 weeks intensive treatment of streptozotocin-diabetic rats with insulin or aldose reductase inhibitors. Insulin was delivered from sustained release insulin implants (Linplant; Møllegaard, Denmark); other diabetic rats received either the aldose reductase inhibitor ponalrestat (ICI Pharmaceuticals, Macclesfield, UK) at 25mg/kg/day by dietary admixture, or imirestat (Hoechst AG, Frankfurt/Main, Germany) at 1 mg/kg/day p.o. Sciatic nerve Doppler flux was measured under non-recovery anaesthesia induced with halothane and then maintained by continuous infusion of alphaxalone (9 mg/ml)/alphadolone (3 mg/ml) (Saffan; Pitman-Moore Ltd, Uxbridge, UK) at a rate of 12 mg/h with removal of the halothane source. Systemic arterial pressure was recorded from a carotid cannula and cardiac rate derived from pulse frequency.

In the time-course study, diabetic sciatic nerve Doppler flux was variable during the two days after streptozotocin injection; from Day 4, when the measurement was 80% of control, fluxes fell steadily and appeared to plateau at 40% of control values after 4 weeks of diabetes. In the second study, treatment with insulin prevented the weight loss and hyperglycaemia of diabetes; neither aldose reductase inhibitor did. Sciatic nerve Doppler flux (mean ± SD in arbitrary units) was reduced by 66% in untreated diabetics (193±62; $p<0.01$ versus controls) compared to controls (435±53); this deficit was prevented by insulin (396±62; $p<0.01$ versus untreated diabetics), partially ameliorated by imirestat (284±97; $p<0.01$ versus controls and $p<0.05$ versus untreated diabetics) and unaffected by ponalrestat (235±80). Both aldose reductase inhibitors normalised nerve sorbitol and fructose levels, indicating pharmacological efficacy against aldose reductase. In neither studies were there were any differences in group mean arterial pressures (for example, mean pressure (mm Hg ± SD) was 114±5.9 in controls and 112±6.4 in untreated diabetics). Heart rates (b/min ± SD) of control (426±97) and insulin-treated diabetic (479±128) animals were higher than those of the remaining diabetic groups (355±84 for untreated diabetics, with similar values for ponalrestat or imirestat treatment). These observations suggest that sciatic nerves of rats with short-term diabetes are markedly ischaemic and that this ischaemia in streptozotocin-diabetes is evident within several days, reaches a plateau by 4 weeks and is maintained for at least 2 months. The findings also indicate that insulin, but not aldose reductase inhibitors at the doses tested, can prevent nerve ischaemia.

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29P REGIONAL HAEMODYNAMIC RESPONSES TO PITUITARY ADENYLATE CYCLASE-ACTIVATING PEPTIDE AND VASOACTIVE INTESTINAL POLYPEPTIDE IN CONSCIOUS RATS: EFFECTS OF L-NAME

S.M. Gardiner, T. Rakhit, P.A. Kemp, J.E. March and T. Bennett. Department of Physiology and Pharmacology, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Although pituitary adenylate cyclase-activating polypeptide (1-27) (PACAP27) shows 68% homology with vasoactive intestinal polypeptide (VIP), Nandha *et al.* (1991) found that PACAP27 was 3 times less potent than VIP in causing hypotension when administered as bolus doses to anaesthetised rats. In order to determine steady-state effects of the peptides, and any possible contribution from nitric oxide (NO), in the present work we assessed haemodynamic responses to 20 min infusions of PACAP27 or VIP, in the absence and presence of L-NAME, in conscious rats chronically instrumented with pulsed Doppler probes and intravascular catheters for continuous monitoring of changes in renal, mesenteric and hindquarters haemodynamics. All surgery was run under sodium methohexitone anaesthesia (40-60 mg kg^{-1}), with the last surgical intervention (catheter implantation) being carried out at least 24 h before experiments were begun. Randomised infusions (1.5, 7.5 and 15 nmol kg^{-1} h^{-1}) of PACAP27 (n=9) or VIP (n=9) were separated by at least 1 h. Experiments in the absence and presence of L-NAME (3 mg kg^{-1} h^{-1}) were run on separate days. In the absence of L-NAME, resting cardiovascular variables in the 2 groups of animals were not different (PACAP27: heart rate (HR), 328 ± 10 beats min^{-1}; mean arterial blood pressure (MAP), 104 ± 2 mm Hg; renal flow (RF), 7.5 ± 1.3 kHz; mesenteric flow (MF), 6.4 ± 0.6 kHz; hindquarters flow (HF), 4.2 ± 0.2 kHz; VIP: HR, 323 ± 6 beats min^{-1}; MAP, 105 ± 2 mm Hg; RF, 7.7 ± 0.9 kHz; MF, 6.9 ± 0.6 kHz; HF, 4.6 ± 0.3 kHz; mean ± s.e. mean). In the presence of L-NAME, the 2 groups also had similar resting cardiovascular values (PACAP27: HR, 252 ± 11 beats min^{-1}; MAP 139 ± 4 mm Hg; RF, 6.0 ± 1.5 kHz; MF, 4.7 ± 0.3 kHz; HF, 2.7 ± 0.2 kHz; VIP: HR, 256 ± 10 beats min^{-1}; MAP, 137 ± 5 mm Hg; RF, 6.0 ± 0.8 kHz; MF, 4.5 ± 0.4 kHz; HF, 3.1 ± 0.5 kHz), but all were significantly different from those in the absence of L-NAME. Table 1 summarises responses to the highest dose of PACAP27 and VIP.

Table: Integrated cardiovascular responses (areas under or curves; AUC, AOC) during 20 min infusions of PACAP27 or VIP(15 nmol kg^{-1} h^{-1}) in the absence and presence of L-NAME. Values are mean ± s.e. mean; RVC, MVC, HVC = vascular conductance in renal, mesenteric and hindquarters vascular bed, respectively.

	PACAP27	+ L-NAME	VIP	+ L-NAME
HR (AUC; beats)	2957 ± 216*	2543 ± 265*	3201 ± 136*	2228 ± 342*[b]
MAP (AOC; mmHg min)	-428 ± 34*	-496 ± 96*	-518 ± 31*	-653 ± 53*[b]
RVC (AOC or AUC; [kHz mm Hg^{-1}]10^3 min)	414 ± 98*	345 ± 60*	-208 ± 48*[a]	60 ± 25[bc]
MVC (AOC or AUC; [kHz mm Hg^{-1}]10^3 min)	276 ± 85*	321 ± 68*	-298 ± 59*[a]	-57 ± 19*[bc]
HVC (AUC; [kHz mm Hg^{-1}]10^3 min)	663 ± 41*	253 ± 53*[a]	996 ± 108*[a]	426 ± 73*[b]

* $P<0.05$ versus baseline (Wilcoxon's test); [a] $P<0.05$ versus PACAP27 alone (Wilcoxon or Mann Whitney U test); [b] $P<0.05$ versus VIP alone (Wilcoxon's test); [c] $P<0.05$ versus PACAP27 + L-NAME (Mann-Whitney test).

In the absence of L-NAME, hypotensive responses to PACAP27 and VIP were not different, contrary to the findings of Nandha *et al.* (1991). However, the hindquarters vasodilator effect of VIP was greater than that of PACAP27, and was accompanied by reductions, rather than increases, in RVC and MVC. L-NAME inhibited the rise in HVC in response to PACAP27 and VIP, indicating an involvement of NO.

Nandha, K.A. *et al.*, (1991). J. Endocrinol., **129**, 69-73.

30P HAEMOGLOBIN CAUSES ENDOTHELIUM-DEPENDENT CONTRACTIONS OF THE PORCINE ISOLATED SPLENIC ARTERY THAT ARE SENSITIVE TO CYCLOOXYGENASE AND LIPOXYGENASE INHIBITORS

T.Y. Lot and V.G. Wilson. Department of Physiology and Pharmacology, Queen's Medical Centre, Nottingham NG7 2UH.

N^G-nitro-L-arginine methyl ester (L-NAME) has been reported to produce substantial endothelium-dependent contractions of the porcine isolated splenic artery by inhibiting constitutive nitric oxide (NO) synthase (Lot *et al.*, 1993). These responses were also reported to be sensitive to agents known to inhibit cyclooxygenase and lipoxygenase, suggesting the involvement of arachidonic acid metabolites. In the present study we used haemoglobin (Hb), a scavenger of NO (Martin *et al.*, 1985), to determine whether inhibition of NO synthase *per se* or removal of NO is important in the genesis of these endothelium-dependent contractions. The susceptibility of Hb-induced contractions to selective cyclooxygenase and lipoxygenase inhibitors has also been examined.

The splenic artery from male or female pigs, obtained from a local abattoir, were dissected out, and refrigerated overnight in modified Krebs-Henseleit solution containing 2% Ficoll. The following day, 5 mm ring segments of the vessels were mounted under a final resting tension of 2.5 g wt. for the recording of isometric tension as previously described (Lot *et al.*, 1993). The endothelium was removed from some segments by inserting a pair of fine forceps into the lumen and gently rolling the preparations on saline-moistened paper tissue.

Hb (0.1-3μM) produced concentration-dependent contractions in endothelium-intact (E+), but not in endothelium-denuded (E-) tissues. The contractions produced by 3μM Hb in E+ segments were 46.7 ± 6.7% of the maximum response to 5-hydroxytryptamine (5-HT: 18.4 ± 1.7 g wt.; n = 5). Administration of 1mM L-arginine slowly reduced established contractions to 3μM Hb by 40.7 ± 15.7% (n = 8) while 1mM D-arginine had no effect (n = 8). Similarly, reversal of established contractions to 100μM L-NAME in E+ segments from the same artery by 1mM L-arginine was 91.0 ± 13.6% (n = 5) while D-arginine had no effect (n = 5). In E+ tissues, contractions to 3μM Hb were inhibited by 87.2 ± 7.0%, 91.3 ± 3.1% and 89.5 ± 3.5% (n = 5 each) by 10μM flurbiprofen, a selective cyclooxygenase inhibitor (Nishizawa *et al.*, 1973), 10μM AA 861, a selective lipoxygenase inhibitor (Yoshimoto *et al.*, 1982) and a combination of 10μM flurbiprofen and 10μM AA 861, respectively.

Our data indicate that Hb produces substantial endothelium-dependent contractions of the porcine splenic artery that are partially inhibited by L-arginine, suggesting that they involve removal of NO. The contractions are also sensitive to inhibitors of arachidonic acid metabolism, as was observed with L-NAME-induced contractions (Lot *et al.*, 1993), suggesting that removal of NO, rather than inhibition of NO synthase *per se*, alters arachidonic acid metabolism, leading to contraction of the splenic artery.

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31P ROLE OF THE CONSTITUTIVE AND INDUCIBLE NITRIC OXIDE SYNTHASES IN THE CARDIOVASCULAR RESPONSE IN SYSTEMIC ANAPHYLAXIS IN THE RAT

Cs. Szabó, C.C. Wu, C. Thiemermann & J. R. Vane, The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ, United Kingdom

An enhanced formation of nitric oxide (NO) by the constitutive and inducible NO synthase (NOS) contributes to the cardiovascular failure in endotoxic (Szabo *et al.*, 1993) and haemorrhagic shock (Thiemermann *et al.*, 1993). Here we study the role of these NOS isoforms in anaphylactic shock in the rat.

Rats were sensitized to ovalbumin (OA) by i.p. injection of OA (180 mg/kg) on days 1, 2 and 3. On day 14, rats were anaesthetized with sodium thiopental (120 mg/kg i.p.) and mean arterial blood pressure (MAP) and heart rate were measured via a carotid arterial cannula. OA (10 mg/kg or 180 mg/kg; i.v.) was administered at time 0. At 5 and (for 10 mg/kg OA) at 120 min after OA injection, the pressor effects of NA (1 μg/kg i.v.) were recorded. Thoracic aortas and lungs were removed from control animals, from sensitized animals (at day 14), and from sensitized animals at 180 min after the initiation of anaphylaxis with OA (10 mg/kg). Isometric tension of aortic rings (n=7-9 in each group) was measured and dose-response curves to NA (10^{-9} - 10^{-6} M) were obtained before and after treatment with the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, $3x10^{-4}$ M for 20 min) in intact and endothelium-denuded rings. Relaxations to acetylcholine (ACh, 10^{-8}-10^{-5} M) were also obtained. Calcium-dependent (constitutive) and -independent (induced) NOS activity was measured in lung homogenates by the conversion of ^{3}H-L-arginine to ^{3}H-L-citrulline (Szabo *et al.*, 1993).

OA (180 mg/kg i.v.) in sensitized rats caused an immediate fall in MAP from 109±13 (control) to 41±5 mmHg ($p<0.01$; n=5) and a reduction of the pressor effects of NA from 32±7 (control) to 15±3 mmHg ($p<0.01$; n=5). L-NAME (1mg/kg, i.v., 10 min prior to OA) prevented the hyporeactivity (from 30±4 to 40±7 mmHg, n=5). The fall in MAP was only slightly affected (from 146±4 to 62±8 mmHg, n=5). Dexamethasone (3 mg/kg i.v., 60 min prior to OA) significantly attenuated the anaphylactic fall in blood pressure (from 117±8 to 73±7 mmHg, n=5), but did not influence the associated hyporeactivity to NA (from 32±7 to 17±5 mmHg, n=5). The smaller dose of OA (10 mg/kg i.v.) in sensitized rats also caused an immediate fall in MAP and reduced the reactivity to NA (from 27±2 to 19±2 mmHg, n=5). The reactivity and the fall in MAP returned to normal over 120 min. Endothelium-intact aortic rings obtained from control rats, OA-sensitized rats or from sensitized rats 180 min after i.v. OA, showed an enhancement of the contractions to NA (10^{-6} M) after L-NAME. However, this enhancement of the contractions was significantly ($p<0.05$) higher in control rings (97±11%) than in rings from sensitized rats (50±12%) or from anaphylactic animals (37±4%), suggesting a reduced basal release of NO due to sensitization. Relaxations to ACh were similar in control rings and in rings from sensitized rats, but were reduced in rings taken at 180 min after i.v. OA. Endothelium-denuded rings from any of the groups exhibited similar contractions to NA, which were unaffected by L-NAME. Interestingly, in the lungs taken from the sensitized animals there was a reduced constitutive NOS activity (from 18±6 to 5±2 pmol/mg/20 min, $p<0.05$; n=6). However, neither sensitization, nor anaphylactic shock induced a calcium-independent NOS.

Thus, sensitization to OA reduces the basal, calcium-dependent NOS activity in the lung and aorta. Anaphylaxis causes (i) an acute release of NO derived from the constitutive NOS, which contributes to the acute vascular hyporeacivity and (ii) an inhibition of the vasodilator responses to ACh *ex vivo*. (This work was supported by a grant of Glaxo Group Research Ltd. C.S. is supported by the Lloyds of London Tercentenary Foundation. C.-C.W. is supported by the NDMC of Taiwan.)

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Thiemermann C. Szabó C., Mitchell J.A. & Vane J.R. (1993) Proc. Natl. Acad. Sci. USA 90, 267

32P LOW DENSITY LIPOPROTEINS ACUTELY INHIBIT EDRF ACTIVITY IN RABBIT AORTA BY A MECHANISM INVOLVING PROTEIN KINASE C BUT APPARENTLY NOT SUPEROXIDE ANIONS

J.A. Smith, M. Evans and R.A. Evans, Departments of Cardiology and Pharmacology and Therapeutics, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN

Endothelium-dependent relaxation is impaired in atherosclerosis, possibly due to increased superoxide anion production contributing to EDRF destruction. Superoxide anion release from 1 cm sections of New Zealand White rabbit aorta with intact endothelium was measured by the superoxide dismutase (60 U ml^{-1}) inhibitable chemiluminescence produced by the luciferin analogue 2-methyl-6-phenyl-3,7-dihydroimidazol [1,2α] pyrazin (CLA; Nakano, 1990). Acute exposure to LDL (1 mg ml^{-1}; 30 min), whether oxidised (LDLox) or native (LDLn), did not significantly increase superoxide anion release compared to untreated tissues (untreated = 355±118.5, n=6; LDLox = 1147.8±366, n=6; LDLn = 1024.8±336, n=6 (mean ± s.e.mean) photon counts sec^{-1}). Pretreatment with the protein kinase C inhibitor Ro 31-8220 (3-[1-{3-amidinothio}propyl]-3-indolyl)-4-(1-methyl-3-indolyl)-1H-pyrrole-2,5-dione methanesulphonate; 10 μM) had no significant effect on superoxide anion production in untreated or LDL-treated tissues (untreated = 355±118.5, n=6; 8220 alone = 803.6±209.3, n=6; 8220+ LDLox = 1027±270, n=6; LDLn = 1140.8±318.4, n=6 photon counts sec^{-1}). Acetylcholine induced relaxation of phenylephrine pre-constricted aortic rings (maximum relaxation 70.6±1.75%, n=8; ED_{50} = $2x10^{-6}$M) was significantly inhibited by pre-treatment with LDLox (maximum relaxation 24.02±0.88%, n=4 $p<0.01$ cf control; ED_{50} x $1x10^{-5}$M, $p<0.01$ cf control), or LDLn (both 1 mg ml^{-1}, 30 min) (maximum relaxation 32.6±3.7%, n=4, $p<0.05$ cf control; ED_{50} = $3x10^{-5}$M, $p<0.01$ cf control). Pretreatment with Ro 31-8220 (10 μM, 20 min) significantly reduced these inhibitory effects: LDLox maximum 60.7±2.7%, n=4, $p<0.01$ cf LDLox alone; ED_{50} = $6x10^{-6}$M, $p<0.01$ cf LDLox alone: LDLn maximum 53.8±3.9%, n=4, $p<0.05$ cf LDLn alone: ED_{50} = $3.5x10^{-6}$M, $p<0.01$ cf LDLn alone). The results demonstrate that acute exposure to LDL significantly inhibits vascular relaxation, through a mechanism involving PKC but apparently not through increased superoxide anion production.

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33P CARDIOVASCULAR EFFECTS OF AN INHIBITOR OF NITRIC OXIDE FORMATION IN THE ANAESTHETISED MINI-PIG: PRESSURE-VOLUME ANALYSIS

A. Gelzer & H. A. Ball (introd. by S. J. Coker). Cardiovasc. Res. Dept., Ciba-Geigy Ltd, CH-4002 Basel, Switzerland

L-NAME (N^G-nitro-L-arginine methylester), an inhibitor of NO synthase, has been widely used to delineate the role of NO, demonstrating it to be an important modulator of vasomotor tone. Indeed, L-NAME causes an increase in aortic blood pressure and a reduction of cardiac output in a variety of species. While the associated increase in afterload might account for the fall in stroke volume, cardiac depression has also been suggested (Klabunde et al., 1991; Gardiner et al., 1990) to be a contributory factor. We therefore used left ventricular (LV) pressure-volume analysis, an approach providing a relatively load-independent index of contractility, to delineate the cardiovascular effects of inhibition of NO synthesis by L-NAME. Male Göttinger mini-pigs (9 - 13.6 kg) were anaesthetised (pentobarbital) & ventilated. Afterload was characterised by the effective arterial elastance (Ea = end-systolic (ES) pressure/stroke volume (SV)), and LV performance by ES elastance (Ees = slope of ES pressure-volume relation following preload reduction; transient balloon occlusion of the vena cava) obtained from pressure-volume loops (Millar & conductance catheter, respectively). Preload was characterised by end-diastolic volume (EDV). In addition, heart rate (HR), cardiac output (CO), mean arterial blood pressure (MABP), pulmonary artery pressure (PAP), pulmonary vascular resistance (PVR), and right atrial pressure (RAP) were determined at baseline and under steady state conditions following intravenous L-NAME (0.3 - 10 mg kg^{-1}) or saline vehicle.

L-NAME mg kg^{-1}	Ea mm Hg ml^{-1}	Ees mm Hg ml^{-1}	EDV ml	SV ml	HR bpm	CO l min^{-1}	MABP mm Hg	PAP mm Hg	PVR mm Hg l^{-1}	RAP mm Hg
baseline	13.4 ± 1.2	5.3 ± 0.5	21.2 ± 2.8	9.2 ± 0.6	101 ± 4	0.92 ± .08	98 ± 7	16.4 ± 2.3	18.2 ± 2.2	1.8 ± 0.3
0.3	15.9 ± 1.6**	6.0 ± 0.6	21.5 ± 2.7	8.5 ± 0.5*	98 ± 5	0.84 ± .08*	109 ± 9**	17.7 ± 1.8	21.5 ± 1.9*	2.2 ± 0.3
1.0	18.5 ± 2.0**	7.7 ± 1.3	22.1 ± 3.0	8.2 ± 0.7*	97 ± 5	0.80 ± .10*	118 ± 7**	21.1 ± 2.3*	27.7 ± 3.4*	3.1 ± 0.5
3.0	21.4 ± 2.2**	9.8 ± 1.7*	21.3 ± 3.0	7.3 ± 0.5**	96 ± 5	0.71 ± .08**	124 ± 6**	24.4 ± 2.9*	35.5 ± 4.9*	3.4 ± 0.3*
10.0	23.5 ± 2.4**	9.2 ± 0.5**	20.5 ± 3.0	6.6 ± 0.4**	96 ± 5	0.64 ± .07**	123 ± 7*	27.3 ± 3.4*	44.9 ± 7.4*	3.6 ± 0.4*

Data are mean ± s.e. mean of 6 animals with * P< 0.05, ** P< 0.01 compared to baseline

Saline-treated animals did not show changes in any measured parameters (data not shown). L-NAME increased blood pressure and caused a dose-related fall in CO, this being accounted for by a decrease in SV without change in HR. The fall in SV could be entirely accounted for by the dose-related increase in Ea (afterload), there being no change in EDV (LV preload). Furthermore there was no evidence of cardiac depression- on the contrary, Ees (contractility) was increased. The effects of L-NAME on the venous system and venous return could not be delineated due to its marked pulmonary effects (increased RAP with increased PVR). These results confirm the importance of NO in modulation of vasomotor tone, and indicate that the reduction of SV following L-NAME is solely due to an increase in afterload. The possibility that NO also modulates contractility, as has been shown *in vitro* (Balligand et al., 1993), warrants further investigation.

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34P INHIBITORY EFFECT OF N^G-NITRO-L-ARGININE METHYL ESTER (L-NAME) ON VASODILATION IN HUMAN SKIN INDUCED BY INTRADERMAL CALCITONIN GENE-RELATED PEPTIDE (CGRP)

P. C. Goldsmith, N. A. Hayes[1], Pauline M. Dowd & J. C. Foreman[1]. Departments of Dermatology and Pharmacology[1] University College London Medical School, London WC1E 6BT.

CGRP is a potent vasodilator. Its mechanism of action is unknown but both endothelial-dependent and -independent mechanisms of CGRP-induced vasodilatation have been described. In the skin, CGRP-containing neurones have been demonstrated by immunohistochemistry. Intradermal injection of CGRP produces an erythema, at the site of injection which is slow in onset but persists for several hours and is not inhibited by local anaesthetic or pretreatment with topical capsaicin (Piotrowski & Foreman, 1986). Nitric oxide (NO) released constitutively via the action of NO synthase on L- arginine is an important mechanism of endothelium-dependent vasodilatation of some blood vessels. L-NMMA inhibits NO synthase by competitive inhibition of its substrate L-arginine and when it is infused into the brachial artery in man L-NMMA produces vasoconstriction (Vallance et al,1989). We have investigated the possibility that CGRP-induced vasodilatation in human skin occurs via NO synthase.

Local ethical committee approval was obtained for the study. In a constant environment chamber at 21°C healthy volunteers (n=8, 3 male, 5 female, mean age 34 [21-55]) underwent intradermal (ID) injections into the volar aspect of the forearm of 25µl each of: normal saline; L-NMMA 1mM; D-NMMA 1mM; CGRP 1µM; CGRP 0.5µM; CGRP 1µM plus L-NMMA 1mM; CGRP 1µM plus D-NMMA 1mM; CGRP 0.5µM plus L-NMMA 1mM. Cutaneous blood flow was measured by laser Doppler flowmetry (LDF) and changes in blood flow were expressed as % change from baseline. The diameter of the erythematous response was measured by planimetry and from this the total area was calculated. Measurements were made before and at 10 and 20 minutes after injection.

L-NAME and D-NAME produced no visible response when injected alone. There was a significant reduction in the area (cm^2) of the erythematous response to CGRP 1µM(from 0.866±0.089 to 0.546±0.055) at 10 minutes (mean ± s.e.mean) and (from 1.09±0.087to 0.794±0.086) at 20 mins ($p<0.05$ Wilcoxen signed rank) and to CGRP 0.5µM (from 0.898±0.198 to 0.376±0.13) at 10 minutes) and (from 0.891±0.142 to 0.481±0.130) at 20 mins ($p< 0.02$) when injected with L-NMMA 1mM but not with D-NMMA.There was a significant reduction in the increase in blood flow (% change LDF) with CGRP 1µM (from 279±30 to 247±12) at 10 mins and (from 289 ± 27 to 229 ± 9) at 20 mins and with CGRP 0.5µM (from 279±30 to 247±12) at 10 mins and (from 279±30 to 247±12) at 20 mins ($p<0.05$) when injected with L-NMMA 1mM but not with D-NMMA.

These results indicate that in skin CGRP-induced vasodilatation is partially reduced by NO synthase inhibition. We have recently demonstrated the presence of constitutive NO synthase in the endothelium of the microvasculature of human skin by immunohistochemistry (Goldsmith, Polak and Dowd: unpublished data). Thus we postulate that the mechanism of CGRP-induced vasodilatation in human skin is in part mediated by NO synthase present in the cutaneous microvascular endothelium.

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35P GROUPS I, II AND III EXTRACELLULAR PHOSPHOLIPASES A_2: SELECTIVE INHIBITION OF GROUP II ENZYMES BY INDOMETHACIN BUT NOT OTHER NSAIDs

Isabel Benito Lobo and J R S Hoult,Pharmacology Group, Kings College, London SW3 6LX, U.K.

The stable extracellular 14kDa PLA_2 enzymes are grouped into three types (gps I,II and 'III') and are implicated in inflammatory diseases as well as in the offensive action of various venoms. Reports indicate that the NSAID indomethacin can inhibit gp II mammalian PLA_2 (Kaplan-Harris & Elsbach, 1980), but the PLA_2 selectivity of this action has not been determined, and the effects of other NSAIDs are not known. We have therefore compared the actions of indomethacin and seven other NSAIDs on the catalytic activity of a gp I PLA_2 (*Naja naja* venom, Sigma), two gp II enzymes (recombinant human synovial PLA_2, gift of Zeneca Pharmaceuticals; rat peritoneal exudate PLA_2 , obtained 15h post-glycogen elicitation of leukocytes) and a 'gp III' enzyme (honeybee venom, Sigma). PBPB (*p*-bromophenacylbromide) was used as a reference standard. Assay of PLA_2 activity utilised ^{3}H-oleic acid-prelabelled *E. coli* membranes as modified from Patriarca et al (1972).

The results for the NSAIDs are shown in the Table. None of the compounds except indomethacin were inhibitory, showing that of the NSAID types studied, PLA_2 inhibition is confined to the indomethacin type. Moreover, under parallel assay conditions, inhibition was selective to the two gp II enzymes: full dose-inhibition studies revealed micromolar IC_{50} values (Table), but >1000 μM against *Naja* and bee venom enzymes. Like PBPB, the inhibition by indomethacin was greater at low calcium concentrations, but was not time dependent.

Inhibition, percent (based on activity of enzyme alone)				
Test substance; at 1 mM	*Naja naja* venom PLA_2	*Apis mellifera* venom PLA_2	Human synovial secretory PLA_2	Rat peritoneal secretory PLA_2
	Group I	Group III	Group II	Group II
Aspirin	0	0	0	0
Sodium salicylate	0	0	0	0
Paracetamol	0	7.3 ± 5.4	0	0
Oxphenbutazone	0	3.1 ± 9.3	0	15.3 ± 6.9
Ibuprofen	0	0	19.9 ± 10.3	11.3 ± 2.7
Flurbiprofen	0.7 ± 2.6	0	15.9 ± 6.0	19.0 ± 7.5
Indomethacin	32.5 ± 9.8 [>1000]	13.9 ± 1.3* [>1000]	89.5 ± 5.9* [35]	94.0 ± 0.4* [28]
Nabumetone	0	0	0	11.3 ± 7.9

Results show mean ± S.E.M. for 3 tests, IC50 values in μM [in brackets]. *Statistically significant inhibition, $P < 0.05$

We conclude that indomethacin possesses an additional anti-inflammatory feature (capacity to inhibit gp II PLA_2 enzymes in a selective manner), that may be relevant both to its clinical spectrum of action and to the design of novel pharmaceutical leads.

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36P COMPARISON OF *IN VITRO* PROPERTIES OF TWO NOVEL ANTI-INFLAMMATORY PLANT LABDANES WITH ASPIRIN, SODIUM SALICYLATE AND INDOMETHACIN

Beatriz de las Heras, *Angel Villar, *José M Vivas and J R S Hoult, Pharmacology Group, Kings College, London SW3 6LX, U.K. and *Faculty of Pharmacy, Complutense University, 28040 Madrid, Spain.

Several anti-inflammatory and gastroprotective substances have been identified in hexane extracts of plants from the genus *Sideritis*, long used for these purposes in traditional Spanish folk medicine. Two such natural products have been identified from *S. javalambrensis*: *ent*-13-*epi*-12α-acetoxy-manoyl oxide (= "manoyl oxide F1") and *ent*-8α-hydroxy-labda-13(16),14-dien (= "labdane F2") (Cabrera et al., 1983; Villar et al., 1993). We have now studied the possible mechanism(s) of action of these two compounds in various *in vitro* tests, comparing them with the NSAIDs aspirin, sodium salicylate and indomethacin, in order to establish if their anti-inflammatory profile is due to actions (A) on the arachidonate cascade, (B) on the generation or scavenging of reactive oxygen species (ROS), or (C) on the activation of rat or human polymorphonuclear leukocytes.

F1 and F2 dose-dependently inhibited generation of both LTB_4 and TXB_2 by ionophore-stimulated rat mixed peritoneal leukocytes; labdane F2 was more potent, causing >90% inhibition of both products at 10^{-4}M; aspirin and indomethacin preferentially inhibited TXB_2 generation (44% at 10^{-4}M and 100% at 10^{-6}M, respectively, sodium salicylate inactive). However, aspirin (500 μM) and indomethacin (50 μM) but not sodium salicylate, manoyl oxide F1 or labdane F2 (all 500 μM) inhibited arachidonate-induced aggregation of human platelets (71.1 ± 4.1%, 75.3 ± 1.3%, respectively) and TXB_2 generation (both 100%) Manoyl oxide F1 and labdane F2 also inhibited human synovial PLA_2 activity (by <15% and 93% at 10^{-3}M) (aspirin, sodium salicylate inactive at 1 mM; indomethacin 54% inhibition at 0.5 mM). Thus these two natural products are capable of inhibiting the generation of pro-inflammatory mediators, albeit at high concentrations, and may do so by interactions at the PLA_2 level. Neither F1 nor F2 caused any actions at 10^{-5}M to 10^{-4}M on the following: β-glucuronidase secretion by A23187-activated human and rat neutrophils; ; scavenging of superoxide anions; generation of superoxide by activated leukocytes; inhibition of chemiluminescence in the xanthine/xanthine oxidase system. However, at 10^{-4}M F1 and F2 caused 15% extra leakage of LDH in A23187-treated cells, suggestive of a mild cytotoxic action at this dose. As expected, the three NSAIDs were also inactive in all of these tests.

We conclude that these two natural products interact with the eicosanoid system, perhaps at the phospholipase level, but do not interfere with the tested leukocyte functions or with reactive oxygen species, and exhibit mild toxicity to leukocytes at high concentrations.

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37P REGIONAL DISTRIBUTION OF CYTOCHROME P450-ARACHIDONATE (P450-AA) METABOLITES IN RABBIT INTESTINE

M.A. Carroll, M. Balazy & C. Macica. Department of Pharmacology. New York Medical College, Valhalla, NY 10595. USA.

In addition to cyclooxygenase (CO) and lipoxygenase (LOX), AA can be metabolized by the cytochrome P450 monooxygenase pathway, a system which is strictly dependent on NADPH. Since intestinal CO and LOX metabolites are differentially distributed and exert effects on intestinal motility, secretion and blood flow, we characterized P450-AA metabolism by the intestinal tract as some metabolites derived via this pathway modify epithelial ion transport and regional blood flow (McGiff, 1991).

Microsomes were prepared from each region of the intestines of NZW male rabbits (n=8); segments of duodenum, jejunum, ileum caecum and colon were excised and freed of mesenteric blood vessels. Microsomes were also prepared from ileal mucosa and muscularis propria (n=4). Microsomes (0.3 mg/ml) were incubated with ^{14}C-AA (7 μM) for 30min at 37°C and metabolites separated using TLC, visualized by autoradiography and the radioactive zones counted.

TABLE 1	Duodenum	Jejunum	Ileum	Caecum	Colon
+NADPH	0.16±0.03	0.78±0.35	1.86±0.64	0.72±0.02	0.48±0.04
-NADPH	0.11±0.01	0.12±0.03*	0.14±0.02*	0.15±0.02*	0.19±0.02*
Boiled	0.08±0.01*	0.12±0.04*	0.09±0.01*	0.15±0.04*	0.11±0.03*

Values are μg AA converted via P_{450}/mg microsomal protein/30min. *P<0.05 compared to incubates with NADPH (1mM).

In the presence of NADPH (Table 1), ileal microsomes exhibited the greatest P450-AA metabolism whereas duodenal microsomes exhibited the least. For jejunal, ileal and caecal microsomes, AA metabolism was reduced in the absence of NADPH, and was unaffected by indomethacin (10μM), a CO inhibitor, and BW755C (50 μM), a dual CO and LOX inhibitor, but was significantly attenuated by the P450 inhibitors, 7-ethoxyresorufin (1μM) and SKF525A (100 μM). However, colonic microsomal activity was inhibited by both P450 and LOX inhibitors. Comparison of AA metabolism between ileal mucosa and muscularis propria microsomes revealed that both regions metabolized AA to products corresponding to NADPH-dependent, P450-AA metabolites. However, there were quantitative differences in P450-AA metabolism between the two regions; mucosal microsomes exhibiting the highest activity (2.2±0.42 μg/mg protein/30min) compared to muscularis propria microsomes (1.08±0.32 μg/mg protein/30min). Analysis of ileal AA metabolites by high pressure liquid chromatography and negative ion chemical ionization gas chromatography-mass spectrometry of pentafluorobenzyl ester, trimethylsilyl ether derivatives revealed products corresponding to mono-hydroxyeicosatetraenoic acids (HETEs). Semi-quantitative analysis showed that 20-; 19-; 18-; 17- and 16-HETEs were present in a ratio of 6.2:3.3:0.3:0.1:0.1, respectively. Further, ileal P450-AA metabolites dilated the isolated, perfused mesenteric bed, as did 19- and 20-HETE, the major ileal AA products. Since 20-HETE affects epithelial ion transport and blood flow, the P450-AA metabolites may make important contributions to intestinal function.

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38P INDOMETHACIN BLOCKADE OF ANGIOTENSIN-(1-7)-STIMULATED PROSTAGLANDIN RELEASE ATTENUATES THE NATRIURETIC RESPONSE OF THE RAT ISOLATED KIDNEY

C.P. Quilley & S.D. Hilchey, (introduced by M.A. Carroll) New York Medical College, Valhalla, New York. 10595. USA

We previously reported (Dellipizzi et al., 1992) that Angiotensin (Ang)-(1-7), a heptapeptide fragment of the metabolism of either Ang I or Ang II, had three major effects in the rat, isolated kidney, i.e., produced an increase in GFR, a substantial natriuresis and diuresis and an increase in urinary concentration of sodium associated with a fall in potassium concentration. As Ang-(1-7) can stimulate release of arachidonic acid and prostaglandins (PG) we tested the hypothesis that PGs participate in the renal actions of Ang-(1-7).

Kidneys were perfused with gassed (95%O2 ,5CO2), Krebs-Henseleit buffer containing oncotic agents and amino acids for six 10 min. clearance periods at a constant pressure of 90 mmHg. Ang-(1-7) was infused (3pmol/ml), with and without indomethacin (INDO, 10μM), and PGs released in ureteral and venous effluents measured by ELIZA. The data for the last period are described.

NDO reduced urinary and perfusate PGs >80%. During Ang-(1-7) infusion, there was a selective increase in urinary 6-keto$PGF_{1\alpha}$ and TxB_2 (indices of PGI_2 and TxA_2, respectively) to 294±77 and 61±4pg/10min versus control values of 134±18 and 42±5 pg/10min. Solely 6-keto$PGF_{1\alpha}$ increased in the perfusate to 101±9 compared to control of 18±8 pg/ml. The effects of Ang-(1-7) on renal function and their modification by INDO are shown in Table 1.

Table 1; Effect of Indomethacin (10μM) on responses of the rat, isolated kidney to ANG-(1-7) (3pmol/ml)

Treatment	UV(μL/min)	U_{Na}(μEq/ml)	U_K(μEq/ml)	$U_{Na}V$(μEq/min)	U_KV(μEq/min)	GFR(ml/min)
Control	36±5	52±10	24±3	1.7±0.2	0.8±1	0.7±0.1
INDO	27±4	46±7	27±5	3.0±0.3	0.7±0.1	0.7±0.1
ANG-(1-7)	136±23*	84±7*	12±1*	11.6±1.6*	1.7±0.3*	1.0±0.1*
ANG-(1-7)+INDO	60±11†	79±6*	22±3	5.0±1.2†	1.3±0.2*	0.9±0.1

* P<0.05 vs control; † P<0.05 ANG-(1-7) vs ANG-(1-7)+INDO. UV, urine volume; U_{Na} and U_K, urinary sodium and potassium concentration; $U_{Na}V$ and U_KV, urinary sodium and potassium excretion rate; GFR, glomerular filtration rate.

INDO halved the four-fold increase in UV and seven-fold increase in $U_{Na}V$ without affecting the increase in U_{Na} produced by Ang-(1-7). In contrast, the increase in GFR was not changed by INDO, nor was the elevation in U_KV altered as U_K was normalized. Thus, Ang-(1-7) induced diuresis and natriuresis may be partially dependent on stimulation of specific PGs, whereas, the increase in GFR and sodium concentration appear to be independent of cyclooxygenase derived arachidonic acid metabolites.

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Keywords: Angiotensin-(1-7), Prostaglandins, Renal Pharmacology

39P AN ANTIBODY TO INTERLEUKIN-5 INHIBITS ANTIGEN-INDUCED AIRWAY HYPERRESPONSIVENESS AND CELL ACCUMULATION IN GUINEA-PIGS

A.A.Y.Milne & P.J.Piper. Department of Pharmacology, The Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN.

Chronic asthma is characterised by bronchial hyperresponsiveness (BHR) which is correlated with increased numbers of eosinophils in bronchoalveolar lavage fluid (BALF). Cytokine-mediated interactions between lymphocytes and eosinophils may be important in the regulation of airway inflammation in asthma. In particular, interleukin-5 (IL-5) has many actions which are specific for eosinophils, and is present in increased levels in BALF from asthmatics (Walker et al. 1992). An antibody to IL-5 (TRFK-5) reduced the airway eosinophilia in a guinea-pig model of airway inflammation (Chand et al. 1992) but the effect on BHR was not studied. We have used TRFK-5 to evaluate the importance of IL-5 and eosinophils in mediating BHR in antigen-induced airway inflammation in the guinea-pig.

Guinea-pigs were sensitised to ovalbumin over two weeks and challenged with an aerosol of ovalbumin on day 14. 24h later, the animals were anaesthetised (midazolam 7.5mg/kg, fentanyl 0.63mg/kg, fluanisone 20mg/kg) and airway resistance (R) in response to acetylcholine (Ach) given i.v. was measured. Lungs were lavaged (40ml buffer) and total and differential counts made of leukocytes in BALF. Eosinophil peroxidase (EPO) in BALF was assayed using a colorimetric assay (Strath et al. 1985). Animals treated in this way were hyperresponsive to Ach (3 fold reduction in EC_{50}), and had increased total cells (3 fold), eosinophils (16 fold) and EPO (4 fold) in BALF. TRFK-5, or control antibody (rat IgG) was given 1h before challenge (1mg/kg i.v., n=5).

Treatment group	Increase in R with 10μg/kg Ach (cmH_2O/l/s)	Number of leukocytes in BALF ($X10^6$) Total	Eosinophils	EPO (units/ml)
Rat IgG	1141±93	35.4±2.4	11.4±2.6	0.22±0.02
TRFK-5	494±96*	21.6±5.1*	2.8±0.9*	0.13±0.02*

Values shown are mean±s.e.mean. *p<0.05, significantly different from Rat IgG.

Control antibody had no effect on cell numbers in BALF, or on BHR compared to untreated animals. TRFK-5 reduced eosinophilia in BALF, and reduced BHR to Ach. These results confirm that IL-5 is a mediator of the eosinophilia and show further that this cytokine is also involved in the development of BHR in this model.

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40P FUNCTIONAL STIMULATION AND CALCIUM OVERLOAD TOXICITY IN PERITONEAL LEUKOCYTES ELICITED IN RATS BY GLYCOGEN OR INTERLEUKIN-1β

Kallistheni Charalambous and J R S Hoult, Pharmacology Group, King's College, London SW3 6LX, U.K.

The macrophage-derived cytokine interleukin-1β (IL-1β) attracts and activates leukocytes at inflammatory foci by direct or indirect mechanisms. We wished to determine whether IL-1β might influence the functional status and responsiveness of peritoneal leukocytes after *in vivo* administration, with particular regard to the susceptibility of these cells to rapid-onset calcium overload toxicity induced by calcium ionophores (Charalambous & Hoult 1992;1993). We have therefore compared the responses of mixed peritoneal leukocytes induced in male Wistar rats treated (A) with 6% oyster glycogen (10 ml i.p., 16h previously and a saline injection at 3h before sacrifice), or (B) with glycogen as above but also with 5000 units recombinant human IL-1β (Genzyme) i.p. at -3h (= "IL supplemented") or (C) with 2500 units IL-1β i.p. 16h previously plus saline at -3h (="IL-induced"). The leukocytes were harvested and tested for superoxide generation, release of β-glucuronidase LDH leakage and LTB_4/TXB_2 generation as described before , using PMA, FMLP and A23187 as stimulants.

IL-induced peritoneal leukocytes were as numerous as those induced by glycogen, but strikingly enriched in eosinophils (10-15% compared to 1-2% in glycogen-elicited animals and 3-6% in IL-supplemented rats), showing that IL-1β acts as a powerful chemoattractant for leukocyte emigration into the peritoneal cavity. Functional responses in the three groups were broadly similar in that patterns of superoxide generation, β-glucuronidase secretion, cytotoxicity (LDH leakage) and eicosanoid generation (LTB_4, TXB_2) showed similar qualitative features, including activation of the respiratory burst by PMA > FMLP ≥ A23187; secretion of β-glucuronidase by A23187 > FMLP >> PMA (inactive); generation of LTB_4 by A23187 > FMLP >> PMA (inactive), but with A23187 displaying EDTA-preventable calcium overload toxicity at 10^{-5}M in all functional responses. However there were some notable differences between the "IL-induced leukocytes" and glycogen-elicited leukocytes, which may reflect the relative predominance of eosinophils in the IL-induced leukocyte population. Superoxide generation in these cells was greater in response to A23187 (6.0 ± 0.3 v. 3.4 ± 0.2 nmol/10 min, P<0.05), but much less in response to FMLP (2.8 ± 0.1 v. 7.0 ± 0.1, P<0.01), implying differences in signal transduction pathways. This could be resolved by studying purified cell populations. Moreover, the IL-induced leukocytes appeared to be more fragile in that they released more LDH and β-glucuronidase under resting conditions (18.2% v 13.5%, and 22.7% v 9.5%). In conclusion, IL-1β does indeed induce leukocyte emigration into the peritoneal cavity but this cell population is different and displays different responsiveness to that induced by glycogen. However, susceptibility of leukocytes to calcium overload toxicity is not ameliorated by IL-1β treatment.

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41P TYROSINE KINASE INHIBITORS BLOCK THE INCREASE IN Ca^{2+} THAT OCCURS ON THE ADHESION OF SINGLE PLATELETS TO COLLAGEN

A. W. Poole, R. Blake, J. Asselin & S.P. Watson, Dept. of Pharmacology, Mansfield Road, Oxford OX1 3QT

Although low concentrations of collagen ($\leq$ 10 μg/ml) induce hydrolysis of phosphoinositides in indomethacin-treated human platelets, this response is not associated with an increase in $[Ca^{2+}]_i$ in platelet suspensions (Watson *et al*, 1985). One explanation for this discrepancy is that a subpopulation of platelets respond to collagen in an asynchronous manner so that the mean fluorescence increase is below the level of detection. We have investigated this possibility in single platelets adhered to collagen using dynamic imaging techniques. In addition, we have investigated the role of tyrosine phosphorylation in the mobilisation of Ca^{2+} by high concentrations of collagen ($\geq$ 30 μg/ml) in platelet suspensions.

$[Ca^{2+}]_i$ was measured in single platelets loaded with the fluorescent Ca^{2+} indicator fura-2 using IonVision software and a Zeiss Axiovert 35 microscope. Fluorescent images were recorded at 510 nm using two excitation wavelengths, 340 and 380 nm and $[Ca^{2+}]_i$ was subsequently calculated from the ratio of the two sets of images. Platelets were suspended in Tyrodes medium containing indomethacin (10 μM) and applied to plastic coverslips coated with collagen. The number of platelets that adhered to the collagen fibrils increased steadily over several min. After an initial delay of between 15 - 60 s, approximately 50 % of the platelets bound to collagen showed a rise in $[Ca^{2+}]_i$ from a basal of 80 ± 13 to 475 ± 42 nM (n = 4) which was maintained for several min.

Experiments were also performed on platelets in suspension using a Perkin-Elmer LS50B spectrofluorimeter. At concentrations of 30 μg/ml or greater, and after an initial delay of 15 - 30 s, collagen (100 μg/ml) increased $[Ca^{2+}]_i$ to 729 ± 48 nM (n = 3) and this response was inhibited markedly by the tyrosine kinase inhibitor α-cyano-4-hydroxy-3,5-diisopropylcinnamide (ST271; 300 μM) to 184 ± 15 nM, or by the relatively non-specific protein kinase inhibitor staurosporine (10 μM) to 136 ± 12 nM. Consistent with the latter observation, we reported previously that staurosporine completely inhibits collagen-induced formation of [^{3}H]inositol phosphates in indomethacin-treated platelets. ST271 (300 μM) also markedly decreased collagen-induced formation of [^{3}H]inositol phosphates to 14 ± 4 % of the control response.

The present results demonstrate a substantial increase in $[Ca^{2+}]_i$ in single platelets that adhere to collagen. The mechanism of this increase may be through a tyrosine kinase mediated pathway which involves activation of phospholipase C.

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42P FPL 66096: A NOVEL, HIGHLY POTENT AND SELECTIVE ANTAGONIST AT HUMAN PLATELET P_{2T}-PURINOCEPTORS

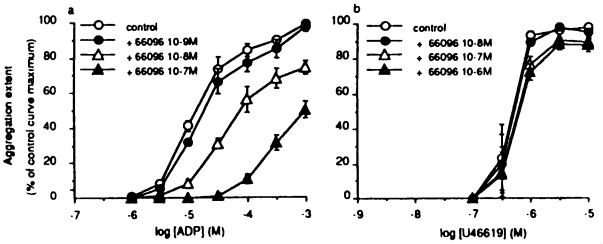
R. G. Humphries, W. Tomlinson, A. H. Ingall*, N. D. Kindon* & P. Leff, Departments of Pharmacology & Medicinal Chemistry*, Fisons plc, Pharmaceutical Division, Research & Development Laboratories, Bakewell Road, Loughborough, LE11 0RH.

Quantitative classification of P_2-purinoceptors has been hampered by the lack of potent and selective antagonists. In the case of the P_{2T}-purinoceptor, which mediates adenosine diphosphate (ADP)-induced platelet aggregation, suramin (Hourani *et al.*, 1992) and analogues of adenosine triphosphate (ATP) (Cusack & Hourani, 1982) have been reported to be antagonists, but these have low affinity and selectivity. In this communication we report studies of the antagonist properties of a novel compound, FPL 66096 (2-propylthio-D-β,γ-difluoromethylene ATP) at human platelet P_{2T}-purinoceptors.

Aggregation was assessed turbidometrically in suspensions of human washed platelets prepared using a modification of the method of Giles *et al.*, (1989). Concentration-effect (E/[A]) curves for the extent of aggregation produced by ADP (10^{-6} - 10^{-3} M, 10 μl) were obtained in 460 μl aliquots of platelet suspension, stirred at 900 rpm, following a 20 min incubation at 37°C. FPL 66096 (10^{-9} - 10^{-7} M) or 30 μl vehicle was added 2 min before ADP. Specificity of the anti-platelet effect was tested against responses to the thromboxane mimetic, U46619 (10^{-7} - 10^{-5} M, 10 μl) rendered ADP-independent by addition of suramin (10^{-4} M) 1 min prior to FPL 66096 (10^{-8} - 10^{-6} M). Selectivity amongst P_2-purinoceptors was assessed using isolated preparations of the rabbit ear artery (P_{2X}) and guinea pig aorta (P_{2Y}) as described elsewhere (O'Connor *et al.*, 1990; Dainty *et al.*, 1992).

FPL 66096 produced concentration-dependent rightward displacements of ADP E/[A] curves (Figure 1a) which, according to Schild analysis, were consistent with simple competition. The Schild slope parameter was 1.05 ± 0.07 and the pK_B estimate, with the former constrained to unity, was 8.74 ± 0.06 (mean ± s.e.mean, n=6). FPL 66096 at a concentration of 10^{-6} M had little effect on E/[A] curves to U46619 in the presence of suramin (Figure 1b). It showed no agonist or antagonist activity at P_{2X}- or P_{2Y}-purinoceptors at up to 3 x 10^{-6} M.

Figure 1: Effect of FPL 66096 on ADP-dependent (a) and independent (b) platelet aggregation (n=4-6)



These results indicate that FPL 66096 is a competitive P_{2T}-purinoceptor antagonist of unprecedented potency and selectivity. As such, it represents a novel pharmacological tool in the classification of P_2-purinoceptors and for investigation of the role of ADP in platelet aggregation.

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43P BIPHASIC DESENSITIZATION OF PROSTACYCLIN RECEPTOR RESPONSIVENESS IN NG108-15 CELLS

R.J. Williams & E. Kelly, Department of Pharmacology, University of Bristol, Bristol BS8 1TD

Prolonged incubation of NG108-15 cells with a prostacyclin agonist leads to heterologous desensitization of adenylyl cyclase, due in part to loss of prostacyclin receptors and Gsα-subunit protein from the cell membrane (Kelly *et al.*, 1990; Adie *et al.*, 1992). Recent work suggests that the prostacyclin receptor and Gsα may co-internalise to some intracellular compartment where down-regulation then takes place (Adie *et al.*, 1992). In this study we have investigated in detail the time course of changes in various signal transduction parameters following prolonged incubation of NG108-15 cells with the stable prostacyclin agonist, iloprost.

Adenylyl cyclase assays in NG108-15 cell homogenates (Kelly *et al.*, 1990), [^{3}H]-iloprost binding (10nM; Adie *et al.*, 1992) and determination of membrane Gsα levels (Williams *et al.*, 1993) were carried out essentially as previously described.

Following incubation of NG108-15 cells with iloprost (1μM) for varying periods of time and washing, the 1μM iloprost stimulated adenylyl cyclase activity as a % of control (100%) was: 1h, 77 +/- 6; 2h, 60 +/- 5; 3h, 53 +/- 4; 4h, 34 +/- 2; 7h, 16 +/- 1 (means +/- s.e.mean, n=4). The decrease between 1 and 2h, 3 and 4h and 4 and 7h, but not 2 and 3h was significantly different ($p<0.05$), indicating a biphasic time course of desensitization. Analysis of the time courses of iloprost induced loss of Gsα-subunit, NaF stimulated adenylyl cyclase activity and [^{3}H]-iloprost binding did not suggest biphasic curves. The $t_{0.5}$ of the initial phase of desensitization (0-3h) was 1.4h, whilst the $t_{0.5}$ for loss of Gsα was 1.1h, that for NaF stimulation 1.4h and that for [^{3}H]-iloprost binding 1.05h (each n=4). This suggests that loss of receptor and G-protein occur at the same time, and account for the 0-3h desensitization due to iloprost. Between 3 and 7h following 1μM iloprost, there was a further profound loss of iloprost responsiveness during which there was no change in NaF stimulation or Gsα levels, although there was a further small decrease in [^{3}H]-iloprost binding (37+/-1% of control at 3h down to 27+/-4% of control at 7h). The second phase of desensitization appears unlikely to be due to activation of protein kinase A, since pretreatment of NG108-15 cells with forskolin (10μM) and Ro20-1724 (100μM) for 1-7h did not decrease iloprost stimulated adenylyl cyclase activity. Furthermore iloprost pretreatment for 1-7h did not affect forskolin (10μM) stimulated adenylyl cyclase, suggesting no change in the function of the catalytic subunit of adenylyl cyclase.

These results indicate that iloprost pretreatment of NG108-15 cells induces an initial desensitization due to loss of prostacyclin receptor and Gsα, and then a further desensitization during which there is no further loss of Gsα. It is unclear at present what induces this second phase of desensitization, but one possibility could include the involvement of a receptor kinase. Thus this model of desensitization of Gs coupled receptors is more complex than previously considered (Adie *et al.*, 1992).

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44P STUDY OF ENDOTHELIN RECEPTOR SUBTYPES MEDIATING CONTRACTION OF RAT AORTA AND TRACHEA USING AGONISTS AND PUTATIVE ANTAGONISTS

M. Kengatharan, T. J. Brown, D. Raeburn and A.G. Roach. Rhône-Poulenc Rorer , Dagenham Research Centre, Rainham Road South, Essex, RM10 7XS.

The selective ET_A receptor antagonist BQ123 (Ihara et al., 1992) has been used to characterise receptors mediating contraction to endothelin-1 (ET-1) in rat aorta (RA) and ET-1 mediated responses in the guinea pig airways (Hay, 1992). In order to further characterise endothelin responses in RA and rat trachea (RT) we have used endothelin receptor agonists ET-1, endothelin-2 (ET-2), endothelin-3 (ET-3), sarafotoxin 6b (STX-6b), sarafotoxin-6c (STX-6c) and [$Ala^{1,3,11,15}$] endothelin-1 (4alaET-1) and the putative selective ET_A receptor antagonists BQ123 and FR139317 (Sogabe et al., 1992).
Rings of RA and RT (3mm) were obtained from male Wistar rats (240-260g) and the endothelium was physically removed from RA. The rings were suspended under resting loads of 2g (RA) and 1g (RT) in 5ml siliconised organ baths containing Krebs' solution at 37°C with protease inhibitors leupeptin (1μM), thiorphan (1μM), bestatin (1μM), bacitracin (750units l^{-1}) and captopril (1μM) and gassed with 95%O_2:5%CO_2. After equilibration for 1h, maximal responses to phenylephrine (PE;3μM) in RA and carbachol (Cch; 10μM) in RT were obtained. Subsequently, responses to PE (30nM; RA) and Cch (300nM; RT) were generated until they became reproducible. Antagonists or vehicle were then added and allowed 30 min incubation period. Each tissue was used for a single agonist concentration-response curve. Antagonist potency (pK_B) against ET-1, ET-2 and STX-6b was estimated in the presence of 1μM antagonist whereas pK_B against ET-3 was estimated using 30nM antagonist. Antagonist potency (pA_2) against ET-1 in the RA was determined using the Schild method (Arunlakshana and Schild, 1959).

Table 1. Potency of BQ123 and FR139317 against various endothelin agonists in the rat aorta. Values are given as mean ± s.e.m, values in brackets indicate no. of animals; analysis of variance (Tukey's method) performed on pK_B values, *$p<0.01$ compared to pK_B against ET-1, ET-2 and STX-6b

	ET-1			ET-2	ET-3	STX-6b
Antagonist	pA_2	Schild plot slope	pK_B	pK_B	pK_B	pK_B
BQ123	6.80±0.09(4)	0.90±0.07(4)	6.85±0.18(4)	7.00±0.17(4)	8.20±0.14(4)*	7.08±0.07(4)
FR139317	6.74±0.08(4)	0.91±0.10(4)	6.71±0.09(4)	6.95±0.09(3)	8.26±0.06(3)*	7.03±0.14(4)

In RA, the order of agonist potency was ET-1=ET-2>STX-6b>ET-3, whereas STX-6c and 4alaET-1 did not contract the tissue up to a concentration of 0.3 μM (ET-1>ET-3 in the absence of protease inhibitors). The putative ET_A selective antagonists used were equipotent and competitive in antagonising contraction to ET-1 (Table 1). However, antagonist potency for BQ123 and FR139317 were significantly greater against ET-3 than the other agonists. In the RT, the rank order of agonist potency was STX-6b=STX-6c=ET-3>ET-1=ET-2>4alaET-1(STX-6c>ET-1=ET-3>4alaET-1 in the absence of protease inhibitors). BQ123 and FR139317 were without effect (at 10μM) against contractions to ET-1, ET-3, STX-6c or 4alaET-1.
These results are consistent with the suggestion that contractile responses to endothelins in the RA are mediated by ET_A receptors (Sumner et al., 1992) and in the trachea by the ET_B receptor subtype (Hay, 1992). However, the disparity in the potency values of the antagonists against ET-3 and the other agonists may imply the existence of various subtypes of ET_A receptors in the RA.

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45P ENDOTHELIN RECEPTOR SUBTYPES IN HUMAN MYOMETRIUM CHARACTERIZED BY BQ123 AND BQ3020

C.R. Bacon*, G. O'Reilly#, I.T. Cameron#, A.P. Davenport* (introduced by M.J Brown). *Clinical Pharmacology Unit, and #Department of Obstetrics and Gynaecology, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QQ.

Immunoreactive endothelin (ET) and its receptors have been detected in human uterine epithelial cells (Cameron *et al.*, 1992 ; Davenport *et al.*, 1991). ET receptors have been found in the myometrium where ET-1 is a potent contractor *in vitro* (Word *et al.*, 1990).

The ET_A selective antagonist BQ123 (Cyclo[D-Asp-L-Pro-D-Val-L-Leu-D-Trp]) and the ET_B selective agonist BQ3020 ([$Ala^{11,15}$]Ac-ET-$1_{(6-21)}$) were used to characterize ET receptor subtypes in human myometrium. Serial 10μm-thick cryostat sections of human myometrium and endometrium were used in saturation binding experiments. Sections were incubated with 8pM-8nM 3-[^{125}Iodotyrosyl^{13}]ET-1 (Amersham International plc) for 2h at 22°C as previously described (Molenaar *et al.*, 1993). Unlabelled ET-1 (1μM) was used to define non-specific binding. In competition experiments, myometrium sections were incubated with 100pM [^{125}I]ET-1 and unlabelled competing ligands BQ123 and BQ3020 in the 100μM-2pM range. Binding data were analysed using the iterative non-linear curve fitting programme LIGAND. In order to detect ET_A and ET_B mRNA, myometrial RNA was extracted by a single-step guanidinium isothiocyanate method. ET_A and ET_B specific primers were used to amplify cDNA by the nested polymerase chain reaction (PCR) (Molenaar *et al.*, 1993).

The saturation assay identified high-affinity endothelin receptors in the myometrium with a dissociation constant (K_D) of 1.19 ± 0.17 nM and Hill slope (nH) of 0.96 ± 0.01 (mean ± s.e.mean for n=3). There was no significant difference (Mann-Whitney test) in receptor affinity in the myometrium compared to the endometrium (K_D, 1.39 ± 0.51nM, nH, 0.98 ± 0.01). Receptor densities (B_{max}) were 37.3 ± 13.9 and 181.3 ± 97.6 (fmol/mg protein) in the myometrium and endometrium, respectively. A heterogeneous population of myometrial receptors was revealed by competition experiments with BQ123 (K_D ET_A, 1.43 ± 0.33nM; K_D ET_B, 39.9 ± 9.06 μM) and BQ3020 (K_D ET_B, 90.1 ± 18.9nM; K_D ET_A, 4.57 ± 0.58μM). PCR amplification of cDNA indicated the presence of both ET_A and ET_B mRNA in the myometrium. ET_A and ET_B primers amplified bands of the predicted sizes of 299 and 428 base pairs, respectively.

The results demonstrate the presence of ET_A and ET_B in human myometrium together with the mRNA encoding both receptor sub-types.

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46P ET_A AND ET_B RECEPTORS MEDIATE THE RELEASE OF EICOSANOIDS FROM THE RAT KIDNEY INDUCED BY ENDOTHELIN/SARAFOTOXIN PEPTIDES

Bruno Battistini, Graham H. Allcock, Timothy D. Warner and John R. Vane. *The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ, U.K.*

In the rat, renal vasoconstrictions induced by endothelin/sarafotoxin (ET/SX) peptides *in vivo* or *in vitro* are mediated by ETA and ETB receptors (Cristol et al., 1993; Wellings et al., 1993) but it is not clear which receptor(s) mediate the release of prostanoids (Trybulec et al., 1991; Stier et al., 1992). Here, using as agonists ET-1 (ETA/B-non-selective) and SX6c (ETB-selective), and as antagonists BQ-123 (ETA-selective) and PD 145065 (ETA/B-non-selective, Cody et al., 1992) we have characterised these receptors.

Male Wistar rats (200-350 g) were anaesthetised (pentobarbitone sodium, 60 mg kg^{-1}), the right renal artery cannulated, the kidney removed, perfused at constant flow (10 ml min^{-1}) with Krebs' buffer (containing 0.1 % BSA) and renal perfusion pressure (RPP) recorded. After equilibration (30 min) antagonist (10^{-6} M) or vehicle (0.1% BSA in 0.9% saline) was infused starting 3 min before and continuing throughout the infusion of cumulative concentrations of either ET-1 or SX6c (10^{-12} to 10^{-9} M), responses to which were allowed to reach plateau. Perfusate was collected for the first 3 min of each infusion and stored at -20°C. Samples were concentrated (20-fold) and the contents of PGI2, PGF2α, and PGE2 measured by radioimmunoassay.

Either ET-1 or SX6c increased RPP (thresholds, 10^{-11} M and $3x10^{-11}$ M), with similar effects at 10^{-9} M (ET-1, 139±7 mmHg; SX6c, 141±20) (n=5-6). BQ-123 or PD 145065 increased the threshold for ET-1 (+BQ-123, $3x10^{-11}$ M; +PD 145065, $3x10^{-10}$ M), and reduced the pressor response to 10^{-9} M ET-1 (+BQ-123, 91±18 mmHg; +PD 145065, 30±11 mmHg)(n=5-6). The increase in RPP induced by SX6c (10^{-9} M, n=6) was unaffected by BQ-123 whereas it was abolished by PD 145056 (n=4). Basal releases of PGE2, PGI2 and PGF2α were 0.32±0.11, 0.5±0.07 and 0.09±0.03 ng/ml, respectively (n=8-11). ET-1 (10^{-9} M) increased these to 2.1±0.64, 4.2±0.5 and 0.79±0.07. These elevations were significantly decreased in the presence of BQ-123 (0.14±0.05*, 0.85±0.05* and 0.13±0.02*) or PD 145065 (1.13±0.27*, 1.42±0.29*, 0.27±0.03*)(n=4-6; *$p<0.05$, t-test). SX6c (10^{-9} M) also stimulated release of PGE2, PGI2 and PGF2α (controls, 2.4±0.38, 2.35±0.15, 0.34±0.03 ng/ml, respectively), which was not affected by BQ-123 (1.58±0.24, 2.65±0.45, 0.66±0.1) but was strongly decreased by PD 145065 (0.01±0.01*, 0.39±0.07*, 0.08±0.01*)(n=4-6).

Thus, in the rat kidney ET-1 is more active than SX6c in producing vasoconstriction and eicosanoid release. The release of eicosanoids induced by ET-1 is antagonised by BQ-123, showing that it is predominantly mediated by ETA receptors. However, SX6c stimulates ETB receptors and produces the same effect. Therefore, in the rat both ETA and ETB receptors mediate renal vasoconstriction and the release of eicosanoids.

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47P ENDOTHELIN-INDUCED VASOCONSTRICTION IN HUMAN ISOLATED VASCULATURE IS MEDIATED PREDOMINANTLY VIA ACTIVATION OF ET_A RECEPTORS

J.J. Maguire & A.P. Davenport (introduced by M.J. Brown) Clinical Pharmacology Unit, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QQ.

Recent reports suggest that in animals ET_A and ET_B receptor activation leads to vasoconstriction both *in vitro* (Ihara et al., 1992; Sumner et al., 1992) and *in vivo* (Gardiner et al., 1992; Cristol et al., 1993). Additionally messenger RNA for both subtypes has been identified in human vascular smooth muscle (Davenport et al., in press). Using subtype-selective ligands we have determined the contribution made by each receptor to endothelin-induced contraction of human isolated blood vessels.

Rings (2mm in width) of endothelium-denuded human saphenous vein (SV), internal mammary artery (IMA) and coronary artery (CA) were mounted under 2g resting tension in organ baths containing Krebs-Henseleit solution (37°C). Cumulative dose response curves (DRC's) were constructed to endothelin-1 (ET-1), ET-3, sarafotoxin S6b and the ET_B-selective agonists sarafotoxin S6c, BQ3020 ([$Ala^{11,15}$] Ac-ET-1(6-21)) and [$Ala^{1,3,11,15}$]ET-1. Peptide responses were expressed as a % of the contraction elicited by 50mM KCl. Additional ET-1 DRC's were constructed in the presence of 0.3-3μM of the ET_A selective antagonist BQ123 (cyclo(D-Asp-L-Pro-D-Val-L-Leu-D-Trp-). ET-1 (10^{-10}-$3x10^{-7}$M)) produced a dose-dependent contraction of SV, IMA and CA with EC_{50} values of 4.0 (2.2-7.2)nM, 6.3 (3.8-10.6)nM and 11.4 (5.5-23.7)nM respectively (geometric mean ± 95% C.I. n≥20). S6b was equipotent with ET-1. ET-3 was much less potent than ET-1 with no responses obtained or the DRC incomplete at 1μM. This difference in potency of the two isoforms provides evidence for the presence of ET_A receptors in these tissues. In agreement increasing concentrations of BQ123 produced a progressive rightward shift of the ET-1 DRC with pA_2 values of 6.4, 7.4 and 7.0 for SV, IMA and CA respectively. Slopes of the Schild regressions were close to unity. No responses were observed to BQ3020 or [$Ala^{1,3,11,15}$]ET-1 (3μM). However S6c contracted 60% of artery and 20% of vein preparations ($EC_{50} \approx 1$nM n≥7). Though potent, the magnitude of the S6c response was less than 50% of that to ET-1 or S6b.

These data suggest that whilst both functional ET_A and ET_B receptors are present on human isolated blood vessels, vasoconstriction is mediated predominantly via the ET_A receptor subtype.

We are grateful to the Theatre staff at Papworth Hospital (UK) for their permission to collect human tissue samples.

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48P STRETCHING, BOTH ACUTELY AND CHRONICALLY, OF ENDOTHELIAL CELLS INCREASES THEIR PRODUCTION OF PROSTACYCLIN AND ENDOTHELIN

Heather Macarthur, Elizabeth G. Wood, *Sean Nair, *Brian Henderson, Timothy D. Warner and John R. Vane. *The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ, & *The Oral Surgery Research Laboratory, Eastman Dental School, Grays Inn Road, London WC1X 8QT.*

Mechanical stretching for periods of up to 360 min increases the release of both prostacyclin (PGI_2) and endothelin-1 (ET-1) from bovine aortic endothelial cells (BAEC) in culture (Macarthur et al., submitted). Here we have investigated the mechanisms producing these effects.

BAEC (primary cultures) were seeded onto culture dishes with flexible bottoms (Petriperm, Bachoffer GMBH, Germany) and allowed to grow to confluence. The medium was removed from the cells and replaced with warmed (37°C) Hepes buffered Locke's solution (pH 7.4) containing 5% BSA. The plates were placed on a cell stretching apparatus, inside an incubator (37°C), and either stretched at 0.2 Hz for 360 min, or in control experiments left unstretched. Buffer from individual plates was collected at 20 or 360 min after the start of the experiment and the amounts of 6-keto-prostaglandin $F_{1\alpha}$ (the stable hydrolysis product of PGI_2) and ET-1 determined by specific radioimmunoassay. Each plate was used for only one time point. Data was compared by ANOVA or student's t-test and $p<0.05$ taken as significant.

The accumulation (20-360 min) of both 6-keto-$PGF_{1\alpha}$ and ET-1 from endothelial cells was increased by stretching ($p<0.05$, Table 1).

collection time	6-keto-$PGF_{1\alpha}$ (ng/ml)		ET-1 (fmol/ml)	
(min)	control	360 min stretch	control	360 min stretch
20	3.8±2.0	3.2±1.1	14.3±1.8	155.5±13.8*
60	11.2±4.1	20.8±12.0	69.7±24.9	237.7±12.7*
120	43.6±11.2	54.3±18.5	126.6±24.6	299.0±15.6*
360	93.7±10.4	196.9±13.8*	157.4±26.2	447.7±45.2*

Table 1. Mediator release from endothelial cells. (*$p<0.05$ difference between control and stretched cells).

Stretching cells for 20 or 60 min caused increases in accumulation of both 6-keto-$PGF_{1\alpha}$ and ET-1 at 360 min that were significantly elevated from unstretched cells ($p<0.05$), but not different from the accumulations produced by stretching for 360 min (n=4 for each).

Thus, stretching of endothelial cells elevates the release both PGI_2 and ET-1. The accumulations at 360 min are due to early effects of stretch for they are mimicked by stretching for periods as short as 20 min.

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49P INVOLVEMENT OF ENDOTHELIAL INJURY, NITRIC OXIDE AND PROSTANOIDS IN THE RAT GASTRIC MICROCIRCULATORY RESPONSES TO ENDOTHELIN-1

J. Lopez-Belmonte* and B.J.R. Whittle. Department of Pharmacology, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS.

Endothelin-1 (ET-1) induces haemorrhagic damage following local infusion to the rat gastric mucosa (Whittle & Esplugues, 1988), which at low doses is accompanied by mucosal hyperaemia (Lopez-Belmonte & Whittle, 1993). This may reflect the release of local vasodilator mediators such as nitric oxide (NO) or prostacyclin from the endothelium (De Nucci et al, 1989), perhaps through activation of ET_B receptors (Masaki et al, 1991) or be secondary to initial endothelial damage. In the present study, the temporal changes in vascular permeability to albumin was used as index of gastric mucosal vascular injury and correlated with changes in mucosal blood flow, determined by laser Doppler flowmetry (LDF). The involvement of prostanoids or NO, in these responses was assessed by the use of the cyclo-oxygenase inhibitor, indomethacin, and the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) respectively.

Rats (240g) were anaesthetised with pentobarbitone and the left gastric artery cannulated for local administration of ET-1. The laser Doppler probe was placed against the surface of the corpus mucosa via a cannula inserted through the fore-stomach (Lopez-Belmonte & Whittle, 1993). Vascular permeability was assessed following administration of [^{125}I]-human serum (0.5μCi kg^{-1} i.v.) by its leakage into the mucosa. Close-arterial infusion of a submaximal dose of ET-1 (5pmol kg^{-1} min^{-1} for 10 min) significantly increased albumin leakage by 17±3; 20±4 and 26±4 μl g^{-1} mucosa (mean±s.emean; n=6; P<0.001; one way ANOVA) at 2.5, 5 and 10 min of infusion, and this was sustained up to 20 min after infusion. By contrast LDF was unaltered at 2.5min, and increased to 25±4% and 72±6% basal at 5 and 10 min of infusion of ET-1 respectively (n=13, P<0.01), and this was sustained up to 20 min after infusion. The LDF response to ET-1 at 10 min was reduced to 53±8% basal (n=12; P<0.05) by pretreatment (10 min) with indomethacin (5mg kg^{-1} i.v.). Furthermore, pretreatment (10 min) with L-NAME (2mg kg^{-1} i.v.) significantly reduced the hyperaemic response to ET-1, (to 36±15% basal, P<0.05) and in combination with indomethacin, abolished this response, revealing a vasoconstrictor response (-14±8% basal, n=7; P<0.05). This combination of indomethacin and L-NAME also abolished the increase in albumin leakage observed after 10 min of the ET-1 infusion (n=5, P<0.001).

These findings indicate that enhanced vascular permeability precedes the increases in blood flow in the gastric microcirculation induced by local infusion of ET-1, suggesting that this hyperaemia may be secondary to endothelial injury. This hyperaemia appears to contribute to the maintenance of the sustained vascular leakage within the microcirculation. Thus, the local release of the endothelium-derived mediators, NO and a prostanoid, probably prostacyclin, which appear to interact to bring about this vasodilatation, may not necessarily be initiated by specific ET-receptor activation, but as a response to direct endothelial perturbation and damage.

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50P *IN VITRO* AND IMMUNOHISTOCHEMICAL STUDIES SUGGEST THAT NITRIC OXIDE, AND NOT VIP, MEDIATES VAGALLY-INDUCED GASTRIC RELAXATION IN THE GUINEA-PIG

K.M. Desai, T.D. Warner, A.E. Bishop*, J.M. Polak* & J.R.Vane. The William Harvey Research Institute, St Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ & *Dept. of Histochemistry, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN

Nitric oxide (NO) mediates vagally-induced relaxation in the isolated stomach of the guinea-pig (Desai *et al.*, 1991). However, vasoactive intestinal polypeptide (VIP) has also been proposed as a neuronal mediator of gastric relaxation in the guinea-pig, where it acts via stimulation of NO production in the muscle cells (Grider *et al.*, 1992). Our aim was to determine the source of NO and investigate any possible link between NO and VIP in mediating vagally-induced gastric relaxation.

Nitric oxide synthase (NOS), the enzyme forming NO from L-arginine, was localized by immunohistochemistry using antibody against the rat brain constitutive NOS (1:5000 dilution). Cross sections of the guinea-pig stomach were studied using the indirect peroxidase anti-peroxidase method (Polak, 1988). Immunofluorescence studies were made on wholemounts (Costa *et al.*, 1980) of the longitudinal muscle-myenteric plexus (LM-MP). Essential positive and negative controls were used. *In vitro* studies were carried out on the isolated stomach of the guinea-pig (Desai *et al.*, 1991). The nonadrenergic, noncholinergic (NANC) relaxant effect of vagal stimulation was studied in the presence of atropine (3 μM) and guanethidine (5 μM). The relaxant effects of vagal stimulation or drugs like VIP were observed after raising the intragastric pressure to 3-4 cm H_20.

Immunohistochemistry of cross sections showed NOS localized mainly to the myenteric plexus ganglia and nerve fibres innervating the circular muscle layer (n = 7). In wholemount preparations of the LM-MP, NOS was localized to nerve cell bodies of the ganglia and nerve fibre varicosities in the muscle layers (n = 6), but not muscle cells. The NOS-positive nerve cell bodies were grouped towards the periphery and had a Dogiel type I morphology, characteristic of motor neurones. These results confirm a neuronal source of NO. In the *in vitro* studies, exogenous VIP (100 nM) induced reproducible relaxations which were unaffected by either tetrodotoxin (TTX, 2 μM, 20 min; control, 30±6% of total volume; + TTX, 33±3%, n=3) or N^w-nitro-L-arginine methyl ester (L-NAME, 100 μM, 20 min), an inhibitor of NOS (control, 29±3%; + L-NAME 30±2%; + L-Arg, 27±2%, n=3). Vagally-induced relaxation was completely inhibited by TTX (control, 35±4%; + TTX, 0 %, n=3), and almost completely inhibited by L-NAME (Desai *et al.*, 1991), which was partially reversed by washout with L-arginine (L-Arg, 2 mM, 30 min) (control, 31±4%; + L-NAME, 7±1%; + L-Arg, 27±1%, n=2).

These results show that the relaxant effects of VIP are not mediated by NO. In addition, the localisation of NOS to nerve cell bodies and fibres supports our view that NO is a major inhibitory transmitter released from nerves by vagal stimulation in the guinea-pig stomach.

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51P THE STIMULATORY AND INHIBITORY EFFECTS OF sCCK-8 IN RAT ISOLATED GASTRIC MUCOSA ARE MEDIATED THROUGH DIFFERENT CCK RECEPTORS

A.J.Baxendale and C.F.Spraggs, Department of Gastrointestinal Pharmacology, Glaxo Group Research Ltd, Ware, Herts, SG12 ODP.

When gastric acid secretion is stimulated by intravenous infusions of sulphated cholecystokinin octapeptide (sCCK-8) in conscious gastric fistula rats, a biphasic dose response curve is obtained. This is modified by the selective CCK_A receptor antagonist devazepide to give a monophasic curve, suggesting that sCCK-8 has both stimulatory and inhibitory effects on acid secretion (Gascoyne, Baxendale and Spraggs, 1992). In this study we have further investigated the effects of sCCK-8 on acid secretion using rat isolated gastric mucosa.

Methods are as previously described (Reeves and Stables, 1987). Briefly, gastric mucosa was removed from female albino rats (60 to 110g) under sodium pentabarbitone anaesthesia, mounted over a small perfusion cup and the serosal side bathed in modified Krebs solution containing indomethacin (2.7μM). The effluent pH of an unbuffered, mucosal perfusing solution, was measured continuously and recorded as H^+ ($nmolmin^{-1}$). Cumulative concentration-response curves were constructed by the addition of agonists to the serosal solution. Antagonists were also added to the serosal solution and allowed to equilibrate for 30 minutes. In experiments to investigate the inhibitory effects of sCCK-8, non-sulphated gastrin-17 (G-17)(1μM) was added to give a maintained secretory plateau and cumulative concentration-effect curves to sCCK-8 were obtained. Values are presented as arithmetic means ± sem or geometric mean with 95% confidence limits from 5 to 8 experiments.

In unstimulated tissues, sCCK-8 and G-17 (1 to 300nM) produced monophasic concentration-response curves with EC_{50} values of 11 (7-16) and 13 (11-16) nM. The selective CCK_B receptor antagonist CI 988 (1μM) produced a parallel rightward displacement in both the sCCK-8 and G-17 concentration-response curves giving a pK_B value of 7.4 against both agonists. Devazepide (10nM) had no effect on G-17 concentration-response curves but produced a 79±21% increase in the sCCK-8 secretory maximum with no significant change in the EC_{50} value (one-way ANOVA, $p>0.05$). In G-17 stimulated tissues, sCCK-8 produced concentration related reductions in acid secretion with an EC_{50} of 9 (5-13) nM. Devazepide (10nM) caused a parallel rightward displacement in the sCCK-8 concentration-response curve giving a pK_B value of 9.1.

In this study in rat isolated gastric mucosa we have shown that both G-17 and sCCK-8 stimulate acid secretion through an action at CCK_B/gastrin receptors. In addition, sCCK-8 is an inhibitor of acid secretion and these effects are mediated through CCK_A receptors.

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52P EVIDENCE THAT RELAXIN ACTS PREDOMINANTLY AT THE LEVEL OF THE PLASMALEMMA IN THE ISOLATED RAT UTERUS

S. J. Hughes & M. Hollingsworth, Smooth Muscle Pharmacology Group, Department of Biological Sciences, Manchester University, Manchester, M13 9PT, UK.

Relaxin inhibition of tension development by the isolated rat uterus may involve direct plasmalemma mechanisms, such as potassium channel opening (KCO), or may be linked via cell-surface receptors to intracellular processes, such as the cAMP pathway (Sanborn *et.al.*, 1980; Downing & Hollingsworth, 1991; Downing *et.al.*, 1992; Hughes *et. al.*, 1992). Tissue bath studies were performed to further assess the mechanism of action of relaxin in comparison with levcromakalim, salbutamol and nifedipine.
Uterine strips from non-pregnant, oestrogen-pretreated rats were mounted for isometric recording. Single, spasmolytic concentration-effect (C/E) curves were constructed by cumulative addition of each relaxant against 4 spasmogens, where the source of calcium (intracellular (Ca_i) or extracellular (Ca_{ex})) was manipulated: 1) oxytocin (0.2 nM) - [OT 0.2] 2) oxytocin (20 nM) + nifedipine (500 nM) - [OT 20] 3) KCl (80 mM) - [80 K] 4) Bay K 8644 (1 μM) (in 10 mM K+-rich PSS) - [BAY K]. The potency (pD_2) of each relaxant against each spasm type was compared to its' potency vs the OT 0.2 spasm, using an unpaired Student's *t*-test (Table 1).

Table 1 Mean pD_2 values ± s. e. m. (n = 6-8) of four relaxants against four spasmogens. * $P < 0.01$, ** $P < 0.001$ vs OT 0.2

	OT 0.2	OT 20	80 K	BAY K
Relaxin	9.15 ± 0.16	7.24 ± 0.25 **	Unmeasurable	7.98 ± 0.23 *
Salbutamol	8.14 ± 0.12	7.98 ± 0.10	6.98 ± 0.14 **	8.48 ± 0.16
Nifedipine	7.86 ± 0.12	Not tested	8.01 ± 0.13	6.74 ± 0.13 **
Levcromakalim	6.58 ± 0.32	4.21 ± 0.17 **	3.95 ± 0.09 **	7.06 ± 0.21

In exhibiting a reduced potency vs OT 20 compared to vs OT 0.2 relaxin was more similar to levcromakalim than salbutamol. If the spasm evoked by OT 20 is largely dependent upon Ca_i then these data indicate that relaxins' action does not directly involve modulation of intracellular events but more likely acts primarily at the plasmalemma. The large reduction in potency of levcromakalim vs 80 K compared to vs OT 0.2 (462 x) is consistent with a KCO action. Similarly, relaxin had a shallower C/E curve vs 80 K compared to that vs OT 0.2 and maximum inhibition was only 53 ± 7% . It may be that relaxin has a partial KCO action. BAY K is dependent upon stimulation of Ca_{ex} influx to elicit spasm. Salbutamol and levcromakalim were equipotent vs BAY K and OT 0.2, whereas nifedipine and relaxin were less potent (by 13 x and 15 x respectively). These data further support a plasmalemmal action of relaxin.
Relaxins' major action seems to be mediated by events occurring at the plasmalemma. However, relaxin may have another, non plasmalemmal-mediated action as it was able to inhibit the spasm evoked by OT 20, which is dependent upon Ca_i only.

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53P EFFECT OF CYCLOPIAZONIC ACID, AN INHIBITOR OF SARCOPLASMIC Ca^{2+}-ATPASE, ON THE FORCE-FREQUENCE RELATION OF GUINEA-PIG ISOLATED ATRIA

N. Yard & H. A. Ball (introduced by S.J. Coker). Cardiovascular Res. Dept., Ciba-Geigy Ltd, CH-4002 Basel, Switzerland

Myocardial contraction is known to be dependent on both extracellular Ca^{2+} influx and Ca^{2+} release from the sarcoplasmic reticulum (SR). Contraction is also dependent on the frequency of stimulation - the so-called force-frequency relation- raising the possibility of frequency dependent changes in Ca^{2+} handling. In order to delineate the functional importance of the SR Ca^{2+}-ATPase in this process we investigated the effect of cyclopiazonic acid (CPA), a potent & specific inhibitor of Ca^{2+}-ATPase (Seidler et al, 1989), on the force- frequency relation of guinea-pig cardiac tissue. Male guinea- pigs (300- 450 g) were anaesthetised (pentothal, 100 mg/kg, i.p.) & left atria dissected. Stimulation was effected by unipolar square wave pulses (5 ms, 2.5 Hz) under isometric conditions in organ baths containing Krebs-Henseleit buffer (5% CO_2/ 95% O_2) at 30°C, in the presence of 1 μM L-propranolol. In the first series of experiments (n = 6) cumulative concentration-response (10 - 100 μM) curves were constructed at a stimulation frequency of 2.5 Hz (table 1). In the second series of experiments (n = 11), frequency relations (figure 1) were constructed before & 1 hour following CPA (30 μM) or an equivalent volume of vehicle (DMSO; 0.2 %). Data are presented as mean ± s.e. mean.

CPA (μM)	Developed tension (g)	time to 95 % relax. (ms)
control	1.8 ± .3	151 ± 11
10	1.3 ± .2 *	202 ± 5 *
30	1.1 ± .2 **	216 ± 7 **
100	0.7 ± .2 **	224 ± 5 **

* P< 0.05; ** P< 0.01 compared to control

Table 1

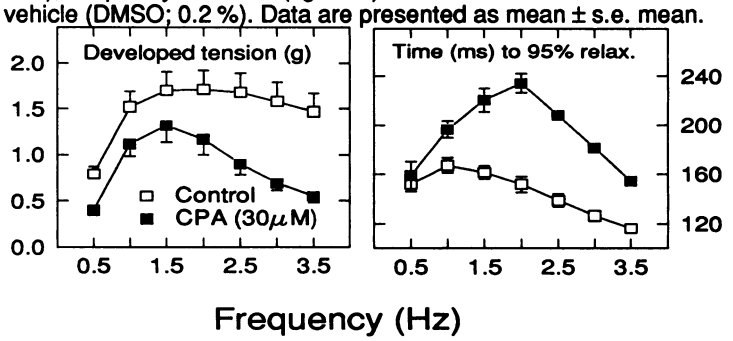

Figure 1:

CPA elicited a concentration-dependent decrease in developed tension & an increase in relaxation time (table 1). The force-frequency relation (figure 1) was shifted downwards (P< 0.01, ANOVA), while the relaxation-frequency relation was shifted upwards (P< 0.001). These results confirm the important role of the Ca^{2+}-ATPase pump of the SR in the normal contraction-relaxation cycle, and suggest a frequency-dependent change in calcium handling with a greater dependence on the SR Ca^{2+}-ATPase pump at higher frequencies.

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54P SOMATOSTATIN RECEPTORS MEDIATING NEGATIVE INOTROPY IN GUINEA-PIG ISOLATED RIGHT ATRIUM

J Dimech, W Feniuk & P P A Humphrey.
Glaxo Institute of Applied Pharmacology, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ.

Somatostatin$_{14}$ (SS_{14}) exerts a negative inotropic effect in guinea-pig isolated atrium (Quirion et al., 1979) but the receptor mediating this response has not been characterised. We have examined the effect of a range of cyclic somatostatin analogues on inotropic activity in guinea-pig right atria, taken from male Dunkin-Hartley guinea-pigs (220-330g). Beating atria were placed in Krebs solution (mM: Na^+ 143.4; K^+ 5.9; Mg^{2+} 0.6; Ca^{2+} 2.6; Cl^- 124.5; $H_2PO_4^-$ 1.2; SO_4^{2-} 0.6; HCO_3^- 25.0; glucose 11.1) at 32°C gassed with 95% O_2/5% CO_2. Changes in developed force were measured from an initial resting tension of 1g. A cumulative concentration-effect (c-e) curve to SS_{14} was constructed on all preparations. Sixty minutes later this was either repeated, or a c-e curve to a test agonist constructed. Equi-active molar ratios (EMR) were measured from the c-e curves at the level (EC_{20}) causing 20% inhibition of developed force.

SS_{14}, SS_{25}, SS_{28} and CGP23996 (cyclo[Ahep-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Tyr-Thr-Ser]) caused a concentration-dependent negative inotropic effect in guinea-pig isolated right atria. SS_{28} was approximately 30 times more potent than SS_{14} (Table 1). Octreotide (0.1nM-3μM) and angiopeptin (1nM-3μM) showed low efficacy producing only a 17.8 ± 2.8% and 7.0 ± 2.1% decrease in force respectively. None of the compounds had any significant effect on heart rate.

Table 1	Agonist	Conc. Range	EC_{20} (nM)	% Inhibition at highest conc.	EMR	n
	SS_{14}	1nM - 3μM	107 (72-160)	46.6 ± 2.6	1.0	12
	SS_{25}	0.1nM - 0.3μM	10.3 (8.6-12.3)	60.0 ± 2.0	0.08 (0.05-0.11)	4
	SS_{28}	0.1nM - 0.3μM	8.0 (2.0-31.9)	55.8 ± 7.0	0.03 (0.05-0.01)	4
	CGP23996	1nM - 10μM	878 (131-5870)	31.5 ± 5.5	4.7 (2.3-8.0)	4

Values are means ± s.e. mean or geometric mean (95% confidence limits) from n experiments.

Following a 30min incubation, angiopeptin (0.1-1μM) produced a concentration-dependent antagonism of the negative inotropic effect of SS_{14} (pA_2 7.47 ± 0.13, slope 0.99 ± 0.12, n=4). Angiopeptin (1μM) did not affect the negative inotropic action of carbachol or cyclohexyladenosine.

The high potency of SS_{28} relative to SS_{14} suggests that the somatostatin receptor mediating negative inotropy in guinea-pig atria is similar to the $SSTR_4$ receptor cloned by O'Carroll et al. (1992). In this functional study, angiopeptin behaved as a somatostatin receptor blocking drug.

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55P EFFECT OF GABAergic STIMULATION ON BASAL STRIATAL PEPTIDE EXPRESSION AND THAT INDUCED BY DOPAMINE ANTAGONIST TREATMENT

J. Jolkkonen, P. Jenner and C.D. Marsden[1]. Parkinsons's Disease Society Experimental Research Laboratories, Pharmacology Group, King's College, London SW3 6LX, U.K. and [1]University Department of Clinical Neurology, Institute of Neurology, The National Hospital, London WC1, U.K.

Dopamine exerts a prominent effect on enkephalin and substance P gene expression in the rat striatum. In addition, GABA and glutamate may be directly or indirectly involved in their regulation (Reimer and Höltt, 1991). The present study further investigated possible GABAergic regulation of striatal peptide expression. First peptide mRNA levels were measured following subchronic GABA receptor stimulation and following administration of the GABA transaminase inhibitor γ-vinyl GABA (GVG). Second the effect of GVG on dopamine antagonists manipulation of peptide expression was studied.

Male Wistar rats were treated with the GABA-A agonist, muscimol (1 mg/kg, i.p., twice daily) or GABA-B agonist, baclofen (4 mg/kg, i.p., twice daily) or GVG (150 mg/kg, i.p., once daily) for 7 days. In addition, GVG was simultaneously administered with the D-1 antagonist, SCH 23390 (0.25 mg/kg, s.c., twice daily) or the D-2 antagonists, eticlopride (2.5 mg/kg, s.c., twice daily) and haloperidol (1 mg/kg, s.c., twice daily) for 7 days. Peptide mRNA levels were assessed from the coronal brain sections (12 μm) using *in situ* hybridization histochemistry as described previously by (Young et al. 1986). After hybridization with ^{35}S-labelled oliconucleotide probes, sections were apposed to film and subsequently analysed by computerized densitometry.

There was a trend for decreased substance P mRNA levels in the striatum following subchronic muscimol (-9.8%), baclofen (-12.3%) or GVG (-13.5%) treatment. In contrast, the abundance of enkephalin mRNA was not affected by any drug treatment. Administration of eticlopride and haloperidol increased enkephalin (50-85%, $p<0.001$) and decreased substance P (25%, $p<0.001$) mRNA levels in the striatum. SCH 23390 had no effect on peptide expression. Altered peptide expression induced by dopamine antagonists was unaffected by the coadministration of GVG.

The data support the importance of D-2 receptor mediated events in the regulation of enkephalin and substance P mRNA levels in the rat striatum. GABA seems to have a minor inhibitory control on peptide expression. No significant GABA-dopamine interaction in peptide expression was observed.

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56P RATIONAL DESIGN OF HIGH AFFINITY NK_1 AND NK_2 TACHYKININ RECEPTOR LIGANDS

S Guard, D C Horwell, W Howson, J Hughes, M C Pritchard, E Roberts, D Rees, K J Watling* and G N Woodruff. Parke-Davis Neuroscience Research Centre, Addenbrooke's Hospital Site, Hills Road, Cambridge, CB2 2QB.

The rational design of non-peptide ligands for peptide receptors demands the identification of the minimally active sequence of the natural peptide ligand (Horwell, 1991). In our initial studies aimed at developing non-peptide tachykinin receptor ligands, we identified the hexapeptide Cam-1666 (LeuMetGlnTrpPheGlyNH$_2$), derived from substance P, as a high affinity NK_2 receptor ligand. This is in agreement with a previous study which described a similar hexapeptide as retaining high affinity for this receptor (McKnight et al 1988). Subsequently, we sought to determine the key amino acid side chains of Cam-1666, which confer affinity for NK_2 binding sites, in order to design lower molecular weight, high affinity NK_2 receptor ligands. A series of peptide analogues have been synthesised and evaluated in tachykinin receptor radioligand binding assays. In addition to yielding a high affinity, low molecular weight NK_2 receptor ligand, these studies also yielded a high affinity, non-peptide NK_1 receptor ligand.

Peptides were tested in radioligand binding assays as follows; NK_1 receptor -[^{125}I]-Bolton Hunter substance P binding (0.1nM) to guinea-pig cerebral cortex membranes (Lee et al 1986), NK_2 receptor - [^{125}I]-iodohistidyl neurokinin A binding (0.1nM) to hamster bladder membranes (Buck and Shatzer 1988) and NK_3 receptor - [^{3}H]-senktide binding (2nM) to guinea-pig cerebral cortex membranes (Guard et al 1990). Data are given as pKi values (arithmetic mean ± SEM, n=3-6).

The relative contribution of each of the amino acid side chains of Cam-1666 (pKi 7.93 ± 0.05) to NK_2 receptor binding affinity was determined by evaluating analogues of this peptide where each amino acid in turn was replaced with L-Ala. This study revealed the primary importance of the Trp and Phe residues and led to the identification of Z-(S)Trp(S)PheNH$_2$ (pKi 5.57 ± 0.05) as a micromolar NK_2 receptor lead. Incorporation of methyl groups along the peptide backbone of Z-(S)Trp(S)PheNH$_2$ gave rise to a series of conformationally restricted analogues, with Z-(S)Trp(R,S)α-MePheNH$_2$ (pKi 6.49 ± 0.10) being the most active of this series. Substitution of the N-terminal protecting group with a 2,3-diOMe moiety, together with the incorporation of a Gly residue into the C-terminus, yielded (2,3-diOMe-Z)-(S)Trp(S)α-MePheGlyNH$_2$ (Cam-2291) which displays a pKi value of 8.86 ± 0.06 versus NK_2 binding sites whilst being 1000-fold less active at NK_1 and NK_3 binding sites. Further studies indicated that Z-(S)Trp(S)PheNH$_2$ also displays micromolar affinity for NK_1 binding sites (pKi 5.38 ± 0.05) and, using a similar strategy to that outlined above, [4-Me]Z-(R)αMeTrpNH(S)CHMePh (Cam-2445) was identified as a high affinity, non-peptide NK_1 receptor ligand, possessing a pKi value of 7.82 ± 0.10 versus NK_1 binding sites whilst being essentially inactive at NK_2 and NK_3 binding sites (pKi values < 5).

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57P SEPTIDE: AN AGONIST OF THE NK_1 RECEPTOR ACTING AT A SITE DISTINCT FROM SUBSTANCE P

L. Pradier*, J. Ménager[1], J. Le Guern, M.D. Bock[1], E. Heuillet[1], V. Fardin[1], A. Doble[1] & J.F. Mayaux, Biotechnology and [1]Biology Departments, Rhône-Poulenc Rorer CRVA, 13 quai Jules Guesde, 94400 Vitry Sur Seine, France.

The hexapeptide septide has been shown to be an agonist as potent as substance P (SP) in eliciting smooth muscle contraction in several in vitro preparations (Wormser et al., 1986) while being a poor competitor of labelled SP binding in rodent brain (Fardin & Garret, 1991). These results as well as other pharmacological data, have suggested the existence of a septide receptor or of a different septide site on the NK1 receptor. We have used rat recombinant NK1 receptor to address this issue.
Rat NK1 receptor was expressed in COS1 cells by transfection, cells plated in 6 or 24 well culture plates and total inositol phosphate (IP) levels were measured by chromatography (Heuillet et al., 1993). SP and septide elicited similar maximal increases in IP levels (4 to 6 fold) in transfected cells with EC_{50} values of 0.05 ± 0.02 nM for SP and 5 ± 2 nM for septide. No additivity of the maximal responses to both agonists was observed and neither agonist evoked any response in sham transfected cells. RP 67580 was a competitive inhibitor of SP response with an inhibition constant (K_B) of 7 ± 2 nM (n = 4) in agreement with displacement studies of [^{3}H]-SP on membranes of transfected cells (Ki value of 10 ± 4 nM). In comparison, septide responses were inhibited by RP 67580 in an uncompetitive fashion with an apparent K_B value of 1.2 ± 0.2 nM (n = 5). In binding studies, septide at concentrations up to 3 µM did not displace [^{3}H]-SP or [^{3}H]-RP 67580 binding on membrane of or on intact transfected cells (see also Hermans et al., 1992).
In conclusion we have demonstrated that septide is a potent agonist of the NK1 receptor but seem to act at a specific subsite different from that of SP. Although not ruling out the existence of selective septide receptor in certain tissues, these results could explain some of the discrepancies with regard to the pharmacological properties of septide. Furthermore, a specific septide site on the NK1 receptor could represent an original pharmacological target.

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58P INHIBITORY FEEDBACK REGULATION OF NK_1 TACHYKININ RECEPTOR SIGNALLING BY PROTEIN KINASE C IN HUMAN UC11 ASTROCYTOMA CELLS

A.J. Barr & S.P. Watson, Dept. of Pharmacology, Mansfield Road, Oxford OX1 3QT

Involvement of protein phosphorylation in the regulation of NK_1-stimulated formation of [^{3}H]-inositol phosphates (IPs) has been investigated in human UC11 astrocytoma cells challenged with the selective agonist [Sar^9,$Met(O_2)^{11}$]-substanceP ([Sar^9,$Met(O_2)^{11}$]SP) in the presence of a range of agents which regulate protein kinases and protein phosphatases.

IPs were measured as described by Barr & Watson, (1993) in the presence of LiCl (10 mM) and data has been expressed as a % ± s.e.mean of the response to [Sar^9,$Met(O_2)^{11}$]SP (1µM) after 30 min. [Sar^9,$Met(O_2)^{11}$]SP (1µM) stimulated a linear accumulation of IPs over a period of 90 min; a 5.0±0.3 (n=26) fold increase in IPs was observed at 30 min. Phorbol dibutyrate (PDBu)(1µM), an activator of protein kinase C (PKC), significantly reduced [Sar^9,$Met(O_2)^{11}$]SP stimulated accumulation of IPs to 47±2% ($p<0.01$) of control levels (100±1%). This effect was not seen in the presence of the selective PKC inhibitor Ro31-8220 (1µM), under which conditions the response to [Sar^9,$Met(O_2)^{11}$]SP was increased to 158±7%. [Sar^9,$Met(O_2)^{11}$]SP and Ro31-8220 alone, gave a similar result, with potentiation of IPs to 158±4%. These results demonstrate that NK_1 receptor activation of phospholipase C (PLC) is under feedback regulation from PKC.

In order to investigate the possible existence of additional protein phosphorylation events that may be involved in the regulation of PLC by NK_1 receptors, we used the relatively nonselective inhibitor of protein kinases, staurosporine, and the inhibitor of protein phosphatases 1 and 2A, okadaic acid. Staurosporine (1µM) increased the response to [Sar^9,$Met(O_2)^{11}$]SP to a level greater than that seen with Ro31-8220 (189±5%) but, in contrast to Ro31-8220, also stimulated formation of IPs to 2.7±0.3 fold of basal levels (equivalent to 54±6% of the [Sar^9,$Met(O_2)^{11}$]SP response). The combined effect of staurosporine and Ro31-8220 on the response to [Sar^9,$Met(O_2)^{11}$]SP was not significantly different from the effect of staurosporine alone. The effect of staurosporine on the response to [Sar^9,$Met(O_2)^{11}$]SP may consist of two components; the increase in basal IPs and inhibition of the PKC feedback pathway. Okadaic acid (1µM) significantly reduced [Sar^9,$Met(O_2)^{11}$]SP stimulated accumulation of IPs to 60±5% ($p<0.01$) and this response was further reduced to 12±4% in the presence of PDBu suggesting that these two agents induce inhibition, in part, through distinct pathways. This is supported by the observation that the inhibitory effect of okadaic acid is only reversed to 83±5% by Ro31-8220, a value that is significantly lower than that seen with Ro31-8220 alone, but is fully reversed by staurosporine to 171±14%. These results suggest that the inhibitory effect of okadaic acid is mediated by potentiation of both PKC and a staurosporine sensitive kinase.

These results demonstrate that protein kinase C plays an important inhibitory feedback role in the regulation of NK_1 tachykinin receptor signalling in human UC11 cells and that basal phospholipase C activity is regulated in an inhibitory manner by an unidentified protein kinase that is sensitive to staurosporine.

We are grateful to Roche for the kind gift of Ro31-8220. AB is an MRC scholar.
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59P [^{125}I]-[MePhe^7]NEUROKININ B MAY LABEL SPECIES VARIANTS OF THE NK_3 BINDING SITE IN GUINEA-PIG AND RAT CEREBRAL CORTEX

P Grimson , S Guard, G N Woodruff and K J Watling. Parke-Davis Neuroscience Research Centre, Addenbrooke's Hospital Site, Hills Road, Cambridge, CB2 2QB, UK.

Binding of the novel NK_3 receptor radioligand [^{125}I]-[MePhe^7]Neurokinin B ([^{125}I]-[MePhe^7]NKB) to NK_3 binding sites present in guinea-pig and rat cerebral cortex membranes has been characterised using methodology previously described by Guard et al (1990) for binding of the selective NK_3 receptor radioligand [^{3}H]-senktide to NK_3 binding sites, with minor modifications. Using senktide (1μM) to define specific binding, binding of [^{125}I]-[MePhe^7]NKB at 20°C was reversible and stable after 2h. Saturation analyses (0.05-3nM) yielded a single population of high affinity binding sites in both tissues possessing the following parameters: guinea-pig - K_d 0.28 ± 0.03nM, B_{max} 338 ± 55 fmol/mg protein; rat - K_d 0.28 ± 0.05nM, B_{max} 42 ± 8 fmol/mg protein (means ± SEM, n=3). The ability of natural and synthetic tachykinins to inhibit binding of [^{125}I]-[MePhe^7]NKB (50pM) was also examined. IC_{50} values were determined, converted to K_i values and data expressed as mean pK_i values ± SEM, n=4-6 (Table 1).

Table 1. pKi values for the inhibition of [^{125}I]-[MePhe^7]NKB binding to NK_3 binding sites in guinea pig and rat cerebral cortex membranes by natural and synthetic tachykinins.

	Guinea pig (pKi mean ± SEM)	Rat (pKi mean ± SEM)
[MePhe^7]Neurokinin B	9.05 ± 0.15	8.90 ± 0.10
Neurokinin B	7.58 ± 0.20	7.22 ± 0.25
[Pro^7]Neurokinin B	6.01 ± 0.19	7.19 ± 0.33
Senktide	7.74 ± 0.08	7.48 ± 0.11
Neurokinin A	6.30 ± 0.08	6.50 ± 0.07
Substance P	6.74 ± 0.15	6.32 ± 0.17
[Sar^9,Met(O$_2$)^{11}]SP	5.56 ± 0.15	< 5.00
(±)CP-96,345	< 5.00	< 5.00
MEN 10,376	< 5.00	< 5.00
L-659,877	< 5.00	< 5.00
SR48968	6.72 ± 0.09	< 5.00

The high affinity of neurokinin B, and the selective NK_3 receptor agonists [MePhe^7]neurokinin B and senktide, as compared with the low affinities of the selective NK_1 receptor antagonist (±)CP-96,345 and the selective NK_2 receptor antagonists MEN 10,376 and L-659,877, suggest that [^{125}I]-[MePhe^7]NKB labels a population of NK_3 binding sites in both tissues. However, the selective NK_3 receptor agonist [Pro^7]neurokinin B displayed an approximate 15-fold higher affinity in the rat as compared with the guinea-pig, whereas the selective nonpeptide NK_2 receptor antagonist SR48968, whilst being inactive in the rat, exhibited a pK_i value of 6.72 in the guinea-pig. In addition to confirming that [^{125}I]-[MePhe^7]NKB is a novel, iodinated radioligand for NK_3 binding sites, these data indicate that the sites labelled in guinea-pig and rat cerebral cortex may be pharmacologically dissimilar and suggest that, as is the case for NK_1 and NK_2 receptors, species-dependent variants of the NK_3 receptor may exist.

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60P INTERACTIONS BETWEEN NEUROPEPTIDE Y AND THE κ-OPIOID SYSTEM IN THE CENTRAL CONTROL OF FOOD INTAKE

P.D. Lambert, J.P.H. Wilding & S.R. Bloom, Department of Endocrinology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London, W12 0NN.

Neuropeptide Y (NPY) and the endogenous κ-opioid receptor ligand dynorphin are hypothalamic neurotransmitters (Abe *et al.*, 1988; Allen *et al.*, 1983) which are of major importance in the control of feeding. Intracerebroventricular (ICV) administration of both NPY or dynorphin produces a robust feeding response in the rat (Morley & Levine, 1983,1984), and altered hypothalamic levels of both peptides and their mRNA's have been found in response to food deprivation. Central pretreatment with the non-selective opioid antagonist naloxone has been shown to reduce NPY-induced feeding (Levine *et al.*, 1990). These observations suggest that NPY and opioid peptides may interact in the central control of food intake. Opioid control of food intake is thought to be predominantly via the κ-opioid receptor, therefore we investigated the effects of ICV injection of the selective κ-opioid receptor antagonist norbinaltorphimine (norBNI) on dynorphin-induced (20μg, ICV) and NPY-induced (10μg, ICV) feeding. ICV pretreatment with norBNI (20μg), a dose which completely attenuated the feeding response to dynorphin, reduced NPY-induced feeding by 67±10% (n=6).

We also studied the feeding response to a 24 hour fast after central injections of norBNI, a selective monoclonal antibody to NPY (NPYAb, 5μl) or norBNI ten minutes before NPYAb. All studies were of crossover design comparing the effects of norBNI with saline and the effects of NPYAb with an antibody to chloroquine which had been prepared in the same way and had no affinity for NPY. Pretreatment with NPYAb 10 minutes before food deprived animals were presented with food reduced their food intake by 32±10% (n=8). The same animals showed a reduction in fast-induced feeding of 34±10% following administration of norBNI. When norBNI was administered 10 minutes before injection of NPYAb there was a 50±8% decrease in the feeding response. These results show that the action of centrally injected NPY to induce feeding involves activation of κ-opioid receptors. However, inactivation of central NPY by NPYAb and pharmacological blockade of κ-opioid receptors with norBNI have an additive effect to reduce fast-induced feeding, suggesting that although the central mechanism inducing feeding following food deprivation involves both NPY and dynorphin, their actions are via separate mechanisms.

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61P THE EFFECT OF ENADOLINE ON IMMEDIATE EARLY GENE EXPRESSION IN THE RAT PAW FORMALIN TEST

E.K.E. Petterson, J.C. Hunter, J.A.Poat & J. Hughes, Parke Davis Neuroscience Research Centre, Addenbrookes Hospital Site, Hills Rd, CAMBRIDGE CB2 2QB

The rat paw formalin test is a model that measures both acute/phasic and late/tonic, continuous, nociception generated by tissue injury (Wheeler-Aceto and Cowan, 1991). It has been reported that Fos-like-immunoreactivity is increased in the spinal cord in this model (Presley *et al.*, 1990) and that enadoline is an effective antinociceptive agent (Wheeler-Aceto and Cowan, 1992). In other models of tissue injury such as gerbil global ischaemia, enadoline has no effect on immediate early genes (IEG's) although it is neuroprotective (McKibbon *et al.*, 1992). The present study examines the effect of enadoline in the formalin nociception model.

Male Wistar rats (70-90g) were administered with a 50µl intraplantar injection of 5% formalin into the left hindpaw and licking behaviour monitored in 5 min periods for 60 min. enadoline (100µg.kg^{-1}), morphine (10mg.kg^{-1}) or saline were administered subcutaneously (2ml.kg^{-1}) 15 min prior to formalin. The animals were killed 5, 30 and 60 min after injury, the spinal cords dissected immediately, frozen in isopentane at -35°C and stored at -70°C until use. Sections (10µm) were examined by *in situ* hybridisation using [^{35}S]-oligonucleotide probes specific for the message under investigation. Autoradiographic images were measured as optical density units using an MCID analyser.

Following formalin injection there was a rapid increase in c-*fos* and NGFI-A mRNA peaking at 60 min. The increases showed a good correlation with the behavioural response and were limited to the lumbar regions of the cord on the ipsilateral side, with strong expression in lamina II decreasing through laminae III-VI. NGFI-A mRNA increased in lamina II from 0.10±0.002 in control to 0.212±0.01 O.D.units in formalin-treated animals, and c-*fos* mRNA from 0.07±0.001 to 0.182±0.013 (3 measurements from 3 animals p<0.001 Mann Whitney U test). The effects of enadoline and morphine on the increases in IEG mRNA and as antinociceptive agents were tested and the results shown in the table.

DRUG PRETREATMENT	% MAX FORMALIN RESPONSE c-fos	NGFI-A	BEHAVIOURAL SCORE (sec) Early Phase (0-10min)	Late Phase (10-45min)	
Saline	100	100	99±7	321±26	The results are from not less than four animals.
Enadoline	73±3*	38±5*	0*	0*	*p<0.001 Mann Whitney U test.
Morphine	N.T.	13±4*	0*	0*	NT = not tested

The results show that enadoline significantly attenuates c-fos and NGFI-A mRNA and completely abolishes the behavioural response demonstrating its analgesic properties.Morphine completely abolished the behavioural response and significantly inhibited the increases in IEGs following formalin. This is in contrast to the inability of enadoline to influence IEG mRNA in other models.

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62P EFFECT OF ENADOLINE ON ISCHAEMIA-INDUCED GLUTAMATE RELEASE

N.J. Hayward, P.K. Hitchcott, A.T. McKnight & G.N. Woodruff, Parke-Davis Neuroscience Research Centre, Addenbrookes Hospital Site, Hills Road, Cambridge, CB2 2QB.

The selective κ-opioid receptor agonist, enadoline (CI-977), has been demonstrated to ameliorate neuronal degeneration in the rat model of focal ischaemia (Hayward *et al.*, 1993). The neuroexcitatory amino acid, glutamate, is implicated in the pathophysiological consequences of brain ischaemia. In this context, enadoline has been shown to inhibit glutamate release in an *in vitro* model of anoxia/hypoglycaemia (Lambert *et al.*, 1991). In the present study, we have investigated the effect of enadoline on glutamate release in the rat model of acute focal ischaemia.

In isoflurane anaesthetized Sprague-Dawley rats (300-375g), guide cannula were stereotaxically implanted into the cerebral cortex and secured in place with skull screws and dental cement. 24 h prior to the ischaemic insult, 2 mm dialysis probes (CMA 11) were implanted. On the study day, the animals were re-anaesthetized and probes perfused with aCSF at 2.5 µl min^{-1}. After a 60 min equilibrium period dialysates were collected at 20 min fractions and amino acid content determined using HPLC with fluorescent detection (Lindroth & Mopper, 1979). Focal ischaemia was induced by permanent occlusion of the left middle cerebral artery (MCAO). Enadoline was administered 30 min prior to MCAO at a neuroprotective dose of 1 mg kg^{-1} s.c. (Hayward *et al.*, 1993). Dialysates were collected for 4 h following MCAO (Table 1). Animals were then perfusion fixed and the extent of ischaemic infarct and probe placement assessed histologically.

Table 1: Dialysate glutamate content (mean ± s.e.mean) from cortex of rats subjected to MCAO.

Treatment Group (n=7)	Time post MCAO (min)					
	-40	0	60	120	180	240
	Dialysate glutamate content (pmol 50µl^{-1})					
MCAO + Saline	23 ± 9	30 ± 12	122 ± 51	93 ± 33	137 ± 55	171 ± 95
MCAO + Enadoline (1 mg kg^{-1})	22 ± 6	18 ± 12	34 ± 12*	24 ± 6*	22 ± 8*	19 ± 7*

* p < 0.05 (Mann-Whitney U-test)

These results indicate that enadoline at a neuroprotective dose of 1 mg kg^{-1} s.c completely antagonises the surge (4-5 fold) in extracellular glutamate in the cerebral cortex observed following focal ischaemia in the rat. The study supports the view that enadoline mediated inhibition of glutamate release could be a major contributory factor to the effectiveness of this compound as a neuroprotective agent.

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63P PHENYTOIN REDUCES THE FREQUENCY-DEPENDENT FACILITATION OF GLUTAMATE-MEDIATED SYNAPTIC TRANSMISSION IN THE RAT ENTORHINAL CORTEX

R.S.G. Jones and A. Dhillon, University Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT.

In vitro studies in rat brain slices have indicated that the entorhinal cortex (EC) is extremely susceptible to epileptogenesis and has the potential to be a primary focus in temporal lobe epilepsies (see Jones and Heinemann, 1991). One factor which may contribute to the predisposition of the EC to epileptogenesis is a short-term enhancement of glutamate mediated excitatory transmission seen during activation of synaptic pathways at relatively low (1-3 Hz) frequencies (Jones, 1991; Jones and Heinemann,1991). In the present experiments we have determined whether the anticonvulsant drug, phenytoin can alter this frequency dependent facilitation of glutamate transmission. 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 (see Jones and Heinemann, 1991) but invariably displayed a fast, AMPA/kainate receptor-mediated EPSP which was succeeded by a slow NMDA receptor-mediated depolarization. In 11 layer V neurones the mean (±SEM) amplitudes of these two components evoked at low frequencies (0.15-0.3 Hz) were 16.6 ± 1.3 mV and 6.5 ± 1.2 mV, respectively. Increasing the stimulation frequency to 1 Hz resulted in a 25 ± 15 % increment in the amplitude of the fast EPSP. Stimulation at 2 and 3 Hz increased the amplitude by 40 ± 16 % and 45 ± 18 %, respectively. Relatively more pronounced facilitation of the NMDA receptor-mediated EPSP was seen, with increases of 38 ± 15 %, 78 ± 23 % and 104 ± 27 % being recorded at 1, 2 and 3 Hz, respectively. All these changes were short-lived and responses returned to control levels within 5-10 sec of restoration of low frequency activation.

In the presence of phenytoin (100 µM) the amplitude of the AMPA-mediated EPSP evoked at low frequency was 14.6 ± 1 mV. This represents a slight, but significant ($p < 0.05$, paired t-test), decrease in amplitude. The NMDA-receptor mediated component was unchanged at 6.1 ± 0.8 mV. The facilitation of both components seen at the three higher frequencies was reduced by the anticonvulsant. In the case of the AMPA-component the % increases in amplitude were 10 ± 5, 21 ± 5 and 22 ± 5 at 1, 2 and 3 Hz. For the NMDA mediated EPSP the values were 10 ± 4, 27 ± 7 and 34 ± 7 %. Although the reduction in facilitation of both components was clear, it only reached statistical significance with the NMDA-EPSP at 2 and 3 Hz ($p < 0.05$, paired t-test).

Thus, although phenytoin did not greatly affect glutamate-mediated transmission in the EC *per se*, it did reduce the short term, frequency-dependent enhancement of both AMPA and NMDA-mediated synaptic responses. It is conceivable that such an action of phenytoin could contribute to its effectiveness against seizures of temporal lobe origin.

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64P CONCURRENT CHRONIC TREATMENT WITH A DIHYDROPYRIDINE Ca^{2+} CHANNEL ANTAGONIST PREVENTS THE EFFECT OF CHRONIC ETHANOL TREATMENT ON TETANUS-INDUCED LONG TERM POTENTIATION

T. L. Ripley and H. J. Little, Department of Pharmacology, The Medical School, University Walk, Bristol BS8 1TD

Chronic ethanol intake is known to cause memory deficits. In the experimental situation, prolonged ethanol treatment has been reported to result in a decrease in long term potentiation, LTP (Durand and Carlen, 1984), a form of synaptic plasticity which has been associated with memory. We have previously shown that concurrent chronic administration of the dihydropyridine calcium channel antagonist, nitrendipine, prevented the development of ethanol tolerance (Dolin and Little, 1989) and ethanol withdrawal signs (Whittington *et al.*, 1990). We have now studied the effects of concurrent chronic ethanol and nitrendipine administration on tetanically-induced LTP.

Male C57 mice, 35-40g, (8-9 per group) received either 24% ethanol v/v (10-14 g/kg/day), 24% ethanol plus nitrendipine 1.77mM, or tap water, for 18 weeks. The intake of ethanol was not significantly different for the former two groups; nitrendipine was removed from the drinking solution 24h before ethanol withdrawal. Hippocampal slices were prepared immediately on withdrawal from ethanol (one slice used per mouse). Extracellular recordings were made from area CA1, with stimulation of the Schaffer collateral commissural pathway. Two tetanic stimuli, 100Hz, 1s, stimulus intensity for half-maximal response, were applied at 4 and 4.5h into withdrawal (i.e. from slice preparation). In the results (Table 1), the single and multiple population spike thresholds (stimulation cut-off = 1000 µA) were measured before the first tetanus and 1.5h after the second tetanus. Population spike heights (same stimulation amplitude as tetani, duration 50 µsec) were normalised to the response amplitude immediately before the first tetanus; "Increase in spike height" in Table 1 was the increase in normalised population spike height from immediately before the first tetanus to 1.5h after the second. "Decrease in potentiation" in Table 1 was the decrease in normalised population spike height from immediately after to 1.5h after the second tetanus.

Table 1	Control	Ethanol	Ethanol + Nitrendipine
Decrease in single spike threshold (µA)	136 ± 9 → 108 ± 5	143 ± 7 → 113 ± 5	147 ± 8 → 117 ± 7
Decrease in multiple spike threshold (µA)	1000 ± 0 → 944 ± 29	944 ± 56 → 637 ± 84**	1000 ± 0 → 772 ± 119
Increase in normalised spike height	78.0 ± 8.7	60.0 ± 10.9	79.3 ± 7.0
Decrease in potentiation	11.6 ± 8.1	33.5 ± 5.2*	13.6 ± 4.4

Values are mean ± s.e.m.. Student's t-test: **$p<0.01$ for extent of decrease; * $p<0.05$ compared with control values

Maintainence of LTP of the population spike, over 90 min from the second tetanus, was decreased in slices prepared after chronic ethanol treatment, compared with controls. This decrease was not seen when nitrendipine was added to the drinking fluid. This suggests that concurrent administration of a dihydropyridine calcium channel antagonist may prevent the adaptive changes during chronic ethanol intake which result in decreases in the maintenance of LTP. It is possible that such treatment may also affect memory loss after prolonged ethanol intake. However, the different pattern seen when a corresponding study was made of calcium-induced LTP (Ripley and Little, this meeting) suggest that the situation may be complex.

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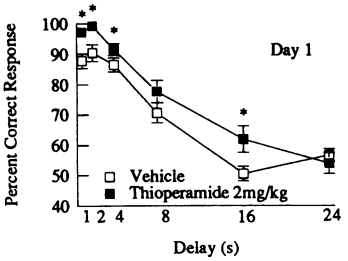
65P ABILITY OF THE SELECTIVE HISTAMINE H_3 RECEPTOR ANTAGONIST THIOPERAMIDE TO IMPROVE SHORT-TERM MEMORY AND REVERSAL LEARNING IN THE RAT

J. Clapham, R.P. Dennes, G.J. Kilpatrick and J.C. Barnes. Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 0DP, U.K.

The existence of neuronal histamine H_3 receptors in the brain is now well established (Arrang et al., 1987). Moreover, thioperamide, a highly selective and potent H_3 receptor antagonist, has been shown to increase the release of acetylcholine from rat entorhinal cortex *in vitro* (Clapham & Kilpatrick, 1992), suggesting that H_3 receptor antagonists may have the potential to improve learning and memory. In the present study we have investigated the ability of thioperamide to improve performance in two models of cognition in the rat.

All experiments used male LH rats maintained on a 23h-food deprivation schedule for the duration of the experiments. Short-term memory was measured using the delayed non-matching to position (DNMTP) task (Dunnett, 1985), using delays of 1,2,4,8,16 and 24s. All animals were initially trained until a criterion of >90% accuracy at the 1 and 2s delays was reached. Animals then received either thioperamide (0.02, 0.2 or 2mg/kg ip bd) or saline (1ml/kg ip bd) for 5 days while training continued daily. In the T-maze, rats were trained to retrieve food from the end of either the left or right arm of the maze. When criterion (5 consecutive correct responses) was reached, treatment with thioperamide (2mg/kg ip bd) or saline (1ml/kg ip bd) commenced and on subsequent test days animals were trained to a serial reversal task. On each day, animals were retrained to criterion (retention test) and then immediately trained to retrieve food rewards from the opposite arm (reversal test). This serial reversal learning procedure was maintained over 4 consecutive days.

Fig: Effect of Thioperamide on DNMTP



In the DNMTP task, thioperamide (2mg/kg ip) significantly improved performance on day 1, see Figure, (* $P<0.05$). This increase in accuracy remained evident on days 2 to 4, although by day 5, performance of vehicle-treated animals improved and performance of vehicle and thioperamide-treated rats was not significantly different. Lower doses were ineffective. In the T-maze task, thioperamide significantly reduced the number of trials to criterion of the reversal task on days 2 and 3, (mean trials to criterion over days 1 - 3: saline 20.4±0.9, thioperamide: 15.4±0.7, $P<0.05$). Thioperamide also improved performance on the retention task on day 3, (Mean trials to criterion following saline 8.6 ±1.0, following thioperamide 6.1 ± 0.5, $P<0.05$).

These results show that thioperamide can improve short-term memory and reversal learning in the rat and implicate the involvement of H_3 receptors in the modulation of cognitive function.

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66P POTENTIATION BY DTT OF HISTAMINE-INDUCED [^{3}H]-IP ACCUMULATION IN RAT CEREBRAL CORTEX

K.W. Young & J.M. Young, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ.

Dithiothreitol (DTT) has been known for some years to potentiate selectively cardiovascular responses to histamine H_1-receptor activation (Fleisch *et al.*, 1973).There is a similar selective potentiation of responses to H_1-agonists in slices of guinea-pig cerebellum and cerebral cortex, where the effect of DTT is to increase H_1-agonist efficacy, such that the concentration-response curve for histamine is shifted to the left by a factor of 6-12 (Donaldson & Hill, 1986). In the course of a study of the effect of various agents on the magnitude of histamine-induced [^{3}H]-inositol phosphate ([^{3}H]-IP) formation in slices of rat cerebral cortex we have observed that DTT has no significant effect on the maximum response to histamine, but causes a dramatic decrease in the EC_{50}.

Cross-chopped slices of rat cerebral cortex were incubated with [^{3}H]-inositol and 10 mM Li^+, with or without DTT, for 30 min at 37°C before addition of histamine and further incubation for 60 min. Incubations were terminated by addition of 10% perchloric acid and [^{3}H]-IPs separated by anion-exchange chromatography. Under these conditions the monophosphate fraction ([^{3}H]-IP_1) constitutes 78 ± 8% (3) of total [^{3}H]-IP. Inhibition by histamine of [^{3}H]-mepyramine binding to homogenates of rat and guinea-pig cerebral cortex was measured at 30°C in 20 mM HEPES buffer, pH 7.5, as described by Treherne *et al.* (1991).

DTT (1 mM) alone caused a small increase in basal accumulation of [^{3}H]-IP_1 in slices of rat cerebral cortex (127 ± 5% of basal, n=7). Histamine stimulated the accumulation of [^{3}H]-IP_1 (204 + 3% of basal, n=31) with an EC_{50} of 60 ± 25 µM (10), the error reflecting an ill-defined maximum response. In the presence of 1 mM DTT the EC_{50} was reduced by a factor of 400 to 0.15 ± 0.02 µM (6). The concentration-response curve for carbachol was not significantly altered by 1 mM DTT. The response to 1 µM histamine was increased from 12 ± 5 to 85 ± 5% of the maximum response to histamine by 1 mM DTT, but cysteine and glutathione (both 1 mM) had no significant effect. Curves of histamine inhibition of [^{3}H]-mepyramine binding to a membrane fraction from rat cerebral cortex (IC_{50} 11 ± 1 µM, n_H 0.56 ± 0.01, n=13) were shifted to the left by a factor of 10 by 1 mM DTT (IC_{50} 1.1 ± 0.1 µM, n_H 0.62 ± 0.02, n=5). In contrast the effect of 1 mM DTT on histamine inhibition of [^{3}H]-mepyramine binding to guinea-pig cerebral cortical membranes was much smaller (IC_{50} 4.3 ± 0.2 µM, n_H 0.79 ± 0.02, n = 2, in the absence of DTT and IC_{50} 3.6 ± 0.1, n_H 0.83 ± 0.02, n = 2, with 1 mM DTT). The effect of DTT on histamine binding the guinea-pig membranes was not changed significantly when measurements were made at 37°C (n=3) or when the membranes were pretreated for 20 min with DTT (n=3).

The very large shift of the curve for histamine-stimulated [^{3}H]IP_1 accumulation caused by DTT in rat cerebral cortical slices reflects, at least in part, the lesser potency of histamine in the absence of DTT than in guinea-pig cerebral cortex or cerebellum. The greater effect of DTT on the IC_{50} for histamine inhibition of [^{3}H]-mepyramine binding to rat compared with guinea-pig cerebral cortical membranes is qualitatively, but not quantitatively, similar to the effect on [^{3}H]-IP_1 accumulation, suggesting that the action of DTT may be at the level of the H_1-receptor.

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67P EFFECT OF ALDEHYDE DEHYDROGENASE INHIBITORS ON BROWN ADIPOSE TISSUE ETHANOL METABOLISM AND LIPOGENESIS IN CBA MICE

M. Al-Qatari and P. V. Taberner, Department of Pharmacology, School of Medical Sciences, University Walk, Bristol BS8 ITD, U.K.

Rothwell and Stock (1984) reported that increased energy expenditure associated with ethanol ingestion may involve brown adipose tissue (BAT) thermogenesis. In 1991, Kortelainen *et al.* reported the presence of alcohol dehydrogenase in BATand suggested that BAT was able to use ethanol as a substrate for thermogenesis. We have reported that chronic ethanol stimulates brown adipose tissue lipogenesis in obese CBA mice (Al-Qatari *et al.*, 1991). The aim of this study was to detect biochemically the presence of aldehyde dehydrogenase in BAT and subsequently assess the role of ethanol as a metabolic substrate for thermogenesis by inhibiting the metabolism of ethanol to acetate, using an aldehyde dehydrogenase (ALDH) inhibitor, cyanamide.
In vivo lipogenesis rates were estimated by measuring the incorporation of ^3H into fatty acids extracted from adipose tissue following i.p. injection of ^3H_2O, using the methods described by Mercer & Trayhurn (1983). ALDH activity was assayed in 0.1M sodium pyrophosphate buffer (pH 9) with 450μM NAD and 10mM propionaldehyde as substrate (Feldman &Weiner, 1975). Mice treated with chronic ethanol were given a drinking solution of 20% w/v ethanol for 4 weeks. Groups of mice treated with cyanamide were gvien an i.p.injection 15mg/kg on 4 consecutive days. Data are means ± S.E.M.(n).
The effect of chronic ethanol treatment on BAT ALDH activity was to significantly increase rates to 1.86±0.56 (10) nM NADH/min/mg protein compared to controls values of 0.78±0.096 (10) ($p< 0.01$, unpaired t-test). Chronic cyanamide treatment inhibited ALDH in both control and ethanol treated activities ($p<0.001$, unpaired t-test) to 0.54± 0.034(8) and 0.43± 0.013(8) respectively. Chronic cyanamide did not signifcantly alter lipogenic rates. BAT lipogenic rates in cyanamide-treated mice, acutely exposed to ethanol (2.5g/kg i.p.) were significantly reduced by 49% to 64.4±13.2 (8) μg atoms H incorporated/hr/g fat free tissue weight ($p<0.01$, unpaired t-test). The effect of chronic cyanamide treatment on lipogenic rates in mice exposed to chronic ethanol treatment showed no significant change 126.5±19.3(8) compared to controls 115.1±9.9(12).
These data indicate that ethanol may be used by BAT as a substrate possibly for thermogenesis. However, the mechanism by which chronic ethanol treatment stimulates thermogenesis is not via its direct oxidation by BAT.

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68P MECHANISMS OF CACHEXIA INDUCED BY T-CELL LEUKAEMIA IN THE RAT

S.Roe, A.L. Cooper[1], I.D. Morris & N.J. Rothwell, Department of Physiological Sciences and[1] North West Injury Research Centre, University of Manchester, Manchester M13 9PT

Cancer cachexia (weight loss) has been ascribed to both a reduction in food intake and/or an increase in metabolic rate. However human and animal studies have yielded conflicting results and previous research has focused largely on solid tumours. The objectives of the present study were to investigate the mechanisms of cachexia in leukaemia induced in PVG rats, (Dibley et al, 1975).

Male (PVG) rats injected with $1x10^6$ leukaemic cells (obtained from a cervical lymph node suspension) showed a rapid increase in white blood cell count, hypertrophy of the spleen (from day 11) and severe morbidity within 17-18 days. Food intake of leukaemic animals declined dramatically from day 12 onwards (day 17: control 20.8 ± 0.2g leukaemic 4.1 ± 3.0g). Body weight gain declined from day 14, and on day 17 was reduced by 67% in leukaemic rats. Weight loss in control animals pair-fed to leukaemic rats was slightly greater (7%) than leukaemic rats but body fat was significantly decreased (18%) and water content increased (14%) in the leukaemic animals compared to pair-fed controls on day 17. Thus body weight measurements significantly underestimated the severity of cachexia.

Resting oxygen consumption (Vo_2) declined in pair-fed animals from day 12, but was significantly elevated ($P<0.001$) in leukaemic animals on days 12-18 by 25% compared to pair-fed and by 7% relative to free-feeding controls. This hypermetabolism was inhibited by administration of either indomethacin (1mg/kg i.p) or DL-propranolol (10mg/kg i.p). In vitro activity of brown adipose tissue (BAT), assessed from mitochondrial GDP binding (Byrant, Rothwell and Stock, 1983), was significantly increased ($P<0.05$) in leukaemic rats by 82% compared to pair-fed and by 70% compared to freely-fed controls. Prior injection of DL-propranolol inhibited the increase in GDP binding activity by 33%.

These results indicate that cachexia associated with T- cell leukaemia in the rat is due largely to hypophagia, but is also accompanied by inappropriately high levels of energy expenditure. The increases in energy expenditure appear to be mediated, at least in part, by prostaglandins and sympathetic activation of thermogenesis in brown adipose tissue (BAT).

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69P INHIBITION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS IN THE HUMAN NEUROBLASTOMA SH-SY5Y BY DESIPRAMINE AND IMIPRAMINE

C. Peers, B. Rana, S.O. McMorn, H.L. Reeve, C.N. Wyatt & P.F.T. Vaughan*, Departments of Pharmacology and *Cardiovascular Studies, Leeds University, Leeds LS2 9JT.

We have previously shown that the nicotinic receptor agonist dimethylphenylpiperazinium iodide (DMPP) evokes release of [^{3}H]noradrenaline (NA) from the human neuroblastoma SH-SY5Y by a mechanism involving depolarization and Ca^{2+} influx through L-type Ca^{2+} channels (Vaughan *et al.*, 1993). Both depolarization and NA release are inhibited by the tricyclic antidepressant (TCA) desipramine (Vaughan *et al.*, 1993). Here, we have investigated the actions of desipramine and imipramine on DMPP-evoked inward currents in voltage-clamped SH-SY5Y cells (Gould *et al.*, 1992).

Cells were perfused with a solution of composition (in mM): NaCl 135, KCl 5, $MgSO_4$ 1.2, $CaCl_2$ 2.5, HEPES 5, glucose 10 (pH 7.4, 21-24°C). Whole cell patch-clamp recordings were obtained from single cells using electrodes filled with (in mM): KCl 117, K-EGTA 11, HEPES 11, $CaCl_2$ 0.1, $MgSO_4$ 2, NaCl 10, ATP 2, (pH 7.2). DMPP was bath-applied either in the absence of TCAs or after 30s pre-exposure to TCAs. All currents were measured for amplitude at their peak and values are expressed as means ± s.e.m. 100μM DMPP evoked inward currents of amplitude 246±31pA (n=13 cells), 357±31pA (n=36) and 655±88pA (n=12) at holding potentials of -40mV, -70mV and -100mV respectively. In the presence of 0.3μM desipramine, these values were reduced to 91±17pA(n=10),136±38pA(n=15) and 199±14pA(n=10) respectively ($p<0.005$ to $p<0.0002$, Student's unpaired t-test). In the presence of 1μM imipramine, currents were also reduced, to 148±32pA (-40mV, n=8, not significant), 172±43pA (-70mV, n=10, $p<0.01$), and 251±39pA (-100mV, n=10, $p<0.001$). The degree to which the TCAs inhibited currents was not altered by using different DMPP concentrations (30μM or 300μM). Concentration - response curves (using 100μM DMPP, holding potential -70mV) yielded IC_{50} values of 0.17μM for desipramine and 1.0μM for imipramine.

TCAs are known to act as antagonists at a variety of receptors (see, for example, Aronstam, 1981). The present study demonstrates that they can also inhibit neuronal nicotinic acetylcholine receptors in human neuroblastoma (SH-SY5Y) cells.

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70P DELIVERY OF MUSCARINIC ACh RECEPTORS TO THE PLASMA MEMBRANE IN UNSTIMULATED AND AGONIST-STIMULATED NG108-15 CELLS

J.A. Koenig & J.M. Edwardson, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ

Neurotransmitter receptors arriving at the plasma membrane may be either newly-synthesized or recycled, and the relative importance of these two routes of delivery appears to vary depending on the state of the cell (Harden *et al.*, 1985). We have characterized the delivery of muscarinic ACh receptors to the plasma membrane in unstimulated and agonist-stimulated NG108-15 cells.

Receptors at the plasma membrane were detected through the binding of the hydrophilic radioligand [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) to intact cells. Non-specific binding was defined by N-methylatropine (1 µM). Receptors at the plasma membrane were irreversibly alkylated using propyl-benzilylcholine mustard (PrBCM; Young *et al.*, 1972), and delivery of receptors to the plasma membrane was then followed through the recovery of [^{3}H]-NMS binding. Recovery was expressed as a percentage of [^{3}H]-NMS binding in control cells.

The binding of [^{3}H]-NMS to muscarinic receptors in NG108-15 cells behaved as a single component of K_D 120 pM and B_{max} 3.63 fmol well^{-1} (≈10^4 sites cell^{-1}). Treatment of the cells with PrBCM caused a rapid ($t_{1/2}$ = 2 min) and complete loss of [^{3}H]-NMS binding. When PrBCM-treated cells were incubated at 37°C for 2h, a partial (32±8%, n=8) recovery of [^{3}H]-NMS binding (of unaltered properties) was seen. When the cells were incubated at 20°C, to block Golgi-plasma membrane transport of proteins (Matlin & Simons, 1983), recovery over 2h in a typical experiment was reduced from 29±5% to 6±4% (n=4). Recovery was also reduced (from 25±2% to 4±1%, n=4) by pre-treatment with the protein synthesis inhibitor cycloheximide (20 µg ml^{-1}). When the cells were exposed to a saturating concentration (0.5 mM) of carbachol for 30 min, [^{3}H]-NMS binding was reduced by 42±3% (n=4), but recovery from PrBCM treatment, in a typical experiment, increased from 25±2% to 38±3% (n=4) of that in control cells. Cycloheximide treatment reduced recovery to 21±2% (n=4).

The virtual abolition of recovery from PrBCM treatment in unstimulated cells by both 20°C incubation and cycloheximide indicates that recovery is almost entirely a result of delivery of newly-synthesized receptors to the plasma membrane, and also that the pool of internalized receptors is normally very small. In carbachol-treated cells, the cycloheximide-sensitive component of recovery is unchanged, indicating that agonist stimulation does not affect the synthetic pathway, but an extra cycloheximide-insensitive component appears, which probably represents recycling from endosomes.

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71P DISRUPTION OFPHOSPHOINOSITIDE SIGNALLING BY LITHIUM IN CHO-CELLS EXPRESSING RECOMBINANT MUSCARINIC M_1-RECEPTORS

Stephen Jenkinson*, R.A. John Challiss & Stefan R. Nahorski, Department of Pharmacology & Therapeutics, University of Leicester, Medical Sciences Building, University Road, Leicester, LE1 9HN.

There is now substantial evidence that the anti-manic agent lithium can disrupt phosphoinositide signalling in brain by its uncompetitive inhibition of inositol monophosphatase and inositol recycling (Nahorski *et.al.*, 1991). Studies in rat cerebral cortex have demonstrated lithium-induced reductions in agonist-stimulated accumulation of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 that appear to result from inositol and phosphoinositide depletion (Kennedy *et.al.*, 1990). The data from such a system are complicated by the heterogeneous cell and receptor population, therefore in order to evaluate these effects more fully we have used a model, Chinese hamster ovary cell (CHO) expressing recombinant M_1 receptors, to examine phosphoinositide metabolism and its disruption by lithium.

CHO-M_1 cells (B_{max} = 816 fmol mg^{-1} protein, [^{3}H]-N-methyl scopolamine binding) grown in 24-well dishes were washed with Krebs-Henseleit buffer and allowed to stabilise for 10 min at 37°C before addition of drugs. Ins(1,4,5)P_3 concentration was determined in perchloric acid extracted cells by a radioreceptor assay (Challiss *et.al.*, 1988).

Addition of a supramaximal concentration of carbachol (1mM) to intact CHO-M_1 cells resulted in a biphasic accumulation of Ins(1,4,5)P_3. Initially, Ins(1,4,5)P_3 levels increased rapidly upon agonist addition, from basal values of 28.8 ± 4.3 pmol/mg protein to 675 ± 37 pmol/mg protein within 10 s. Over the next 50 s concentrations fell to 238 ± 10 pmol/mg protein. A secondary rise then followed reaching a plateau (421 ± 19 pmol/mg protein) after 10 min. Lithium addition (5mM) at zero-time had no significant effect on the initial peak of Ins(1,4,5)P_3 mass. However, a decrease in the plateau phase of Ins(1,4,5)P_3 production was observed with a significant reduction found at 10 min. Lithium alone (10mM) had no effect on Ins(1,4,5)P_3 basal values, however, a concentration-dependent (0.1-10mM) decrease in carbachol-stimulated Ins(1,4,5)P_3 mass (at 20 min) was observed with lithium (IC_{50} = 0.5mM). Exogenous inositol, added 30 min prior to drug addition at the desired concentration (0.1-100 mM) was shown to reverse the inhibition of carbachol-stimulated Ins(1,4,5)P_3 produced by lithium (5mM) in a concentration dependent manner (EC_{50} = 3mM). Pretreatment of the cells for 30 min with 10mM inositol prevented the inhibition of Ins(1,4,5)P_3 production by lithium over the entire time course examined (30 min).

These data strongly support the inositol depletion hypothesis of lithium action in a model cell line expressing recombinant M_1 receptors. It is anticipated that this simple system will allow quantitative estimates of lithium's action and its influence on Ca^{2+} signalling to be assessed in mechanistic detail.

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72P

N.Watson & R.M.Eglen, Institute of Pharmacology, Syntex Discovery Research, 3401 Hillview Avenue, Palo Alto, CA 94303, U.S.A.

In tracheal smooth muscle, the relaxant potency of isoprenaline is higher in tissues pre-contracted with leukotriene D_4 than with muscarinic agonists (Torphy, 1984). One explanation for this is that muscarinic agonists, in addition to causing contraction by muscarinic M_3 receptor activation, may concurrently inhibit ß-adrenoceptor-mediated elevations in adenylyl cyclase activity by M_2 receptor activation (Fernandes *et al.*, 1992). The aim of the present study was to characterize the role of M_2 receptors in the modulation of relaxant responses to isoprenaline in guinea-pig isolated trachea.

Experiments were conducted using tracheal strips prepared from male, Hartley guinea-pigs (250-350g), suspended under 1g tension in oxygenated, modified Krebs solution (pH 7.4, 37°C) containing indomethacin (1µM), tetrodotoxin (1µM) and corticosterone (30µM). All values quoted are mean ± s.e.mean. In the first series of experiments, the effect of methoctramine (a selective M_2 receptor antagonist) on relaxant responses to isoprenaline was studied in tissues pre-contracted to 3g with the non-selective muscarinic agonist, (+)*cis*-dioxolane (2µM). The concentration-response curve to isoprenaline was shifted to the left, in a parallel fashion, in the presence of methoctramine at 0.3, 1.0 and 3.0µM, resulting in potencies (EC_{50}) of 19.1 ± 4.5, 14.5 ± 3.1 and 13.6 ± 3.6 nM, respectively. These values were significantly ($P < 0.05$) different from values obtained in the absence of methoctramine (32.2 ± 4.3nM).

In a second series of experiments the relaxant potency of isoprenaline was investigated in preparations pre-contracted using either SDZ ENS 163 (thiopilocarpine, Enz *et al.*, 1992) or (+)*cis*-dioxolane. SDZ ENS 163 was shown to be a muscarinic M_2 receptor antagonist in the guinea-pig paced left atria preparation (-log K_B=5.9 ± 0.2, n=6) and a partial agonist at muscarinic M_3 receptors mediating contraction of guinea-pig trachea (EC_{50}=1.0 ± 0.1µM). In contrast, (+)*cis*-dioxolane was a full agonist in both preparations (EC_{50}=33.7 ± 6.5nM and 8.1 ± 1.6nM, respectively).The relaxant potency of isoprenaline in preparations pre-contracted to 3g with either SDZ ENS 163 or (+)*cis*-dioxolane was 3.7 ± 0.3 nM and 49.4 ± 3.2nM, respectively. In preparations pre-contracted to 2g with these agonists the relaxant potency of isoprenaline was significantly ($P < 0.05$) enhanced (1.1 ± 0.3 and 6.2 ± 1.1nM, SDZ ENS 163 and (+)*cis*-dioxolane respectively).

These data suggest that muscarinic M_2 receptor antagonism, by methoctramine, significantly enhances the relaxant potency of isoprenaline in tissues pre-contracted by (+)*cis*-dioxolane. The relaxant potency of isoprenaline, however, appears to be not only dependent on the level of initial contracture, but also upon the degree of M_2 receptor agonism/antagonism. These results support data showing that inactivation of M_2 receptor-mediated inhibition of adenylyl cyclase by pertussis toxin pretreatment augments ß-adrenergic relaxation in trachea pre-contracted with muscarinic agonists (Mitchell *et al.*, 1993).

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73P INTERACTIONS BETWEEN LIPOCORTIN-1 AND THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS

Allan W. Sudlow, Frank Carey[1] & Nancy J. Rothwell. Dept of Physiological Sciences, University of Manchester, Oxford Rd., Manchester, M13 9PT, U.K.. [1]Zeneca Pharmaceuticals, Alderley Park, Macclesfield, SK10 4TG, U.K..

Lipocortin-1 (LC-1) is a glucocorticoid-inducible protein known to be present in rat tissues involved in the HPA axis. Recent evidence indicates that LC-1 may inhibit the synthesis, release or action of corticotrophin-releasing factor (CRF).

In the rat, a significant rise in plasma adrenocorticotrophic hormone (ACTH) concentration was observed in response to central (8 μg i.c.v.) or peripheral (45 μgkg^{-1}) administration of CRF (i.c.v.: CRF 63-284 (205) $pgml^{-1}$; vehicle 9-14 (9) $pgml^{-1}$ i.v.: CRF 160-224 (203) $pgml^{-1}$; vehicle 19-22 (19) $pgml^{-1}$, values are range (median); n= 5 or 6; $P< 0.02$, Mann-Whitney). The magnitude of this response was significantly reduced by co-administration of a fragment of recombinant human LC-1 (frh LC-1, Zeneca., UK) at 1.3 μg i.c.v. or 140 μgkg^{-1} i.v. (LC 1-188 & CRF i.c.v 9-128 (38) $pgml^{-1}$ i.v. 19-118 (38) $pgml^{-1}$; n = 5 or 6; $P< 0.05$) but not by peripheral administration of 140 or 280 μgkg^{-1} full sequence rhLC-1 (Biogen Inc., USA).

Primary cultured rat anterior pituicytes and an immortalised mouse anterior pituitary cell line (AtT20) were used to investigate interaction of LC-1 and CRF at the level of the pituitary. The CRF-stimulated (10^{-7}M) release of ACTH from AtT20 cells was not inhibited by co-incubation with either frhLC-1 or full sequence rhLC-1 over the range 10^{-9} to 10^{-7}M . For example, after 180 min incubation period, the CRF (10^{-7}M) stimulated release of ACTH from 3 x 10^6 AtT20 cells (CRF 39 $\pm$ 1; control 22 $\pm$ 1; mean $\pm$ s.e.mean pg ml^{-1}; n=8) was not significantly ($P>0.05$; 2-way ANOVA) altered by co-incubation with 10^{-7}M frhLC-1 (CRF & frhLC-1 36 $\pm$ 3; control 19 $\pm$ 1 $pgml^{-1}$). Experiments with primary rat pituicytes yielded similar results. Dexamethasone (DEX) (10^{-6} or 10^{-7}M) suppression of basal ACTH release from AtT20 and rat corticotroph cells was not significantly altered by anti-LC-1 polyclonal antiserum (Zeneca, U.K.) or monoclonal antibody (Zymed, USA) at various dilutions over 24h. For example, the suppression of ACTH release seen after incubation of 2 x 10^6 AtT20 cells with 10^{-6}M DEX for 24h was not significantly ($P>0.05$) affected by co-incubation with a 1:1000 dilution of either anti-LC-1 monoclonal antibody (MoAb) or control mouse IgG (CON) : vehicle & CON 60 $\pm$ 1; DEX & CON 40 $\pm$ 1; vehicle & MoAb 59 $\pm$ 2; DEX & MoAb 41 $\pm$ 3; mean $\pm$ s.e.mean pg ml^{-1}; n=6. Over a similar time period (>18h), DEX induction of LC-1 in these cells was observed and qualitatively estimated by immunocytochemistry (rat primary pituicytes) and immunoblotting (AtT20; average of 2-fold increase in immunoreactive LC-1 compared with control incubates; n=10).

Thus, although frhLC-1 did inhibit the elevation in plasma ACTH levels induced by CRF in the rat *in vivo*, *in vitro* results indicate that this interaction is probably not at the level of the pituitary.

74P THE EFFECT OF DEXAMETHASONE ON LIPOPOLYSACCHARIDE-INDUCED DISSEMINATED INTRAVASCULAR COAGULATION IN THE ANAESTHETISED RAT

A.J. Ford and D.J. Longridge. Department of Pharmacology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS, U.K.

Dexamethasone (DEX), may be used clinically as adjunctive, supportive therapy to treat the symptoms of septic shock (Lefer and Spath, 1984). Whilst the effects of DEX on the vascular components of systemic bacterial infections have been characterised, little attention has been given to other indices of sepsis such as disseminated intravascular coagulation (DIC). This syndrome is an indication of the hypercoagulable state which may be induced by systemic bacterial infection, and may lead to multiple organ failure, and/or septic shock. The aim of the present study was to elucidate the actions of DEX in an anaesthetised rat model of lipopolysaccharide (LPS)-induced DIC, concentrating specifically on changes in white blood cell (WBC) and platelet count. Selected organs were analysed for accumulation of ^{125}I-labelled fibrin, expressed as a deposition ratio (DR) compared to final blood ^{125}I levels. DIC was induced by a 10 mg/kg/h i.v. infusion (1 h) of LPS from *E.coli*. Animals were killed 4 h after the end of the LPS infusion. Treated animals received DEX as a 3mg/kg i.v. bolus 90 min prior to the start of the LPS infusion, these animals were compared to controls which received a bolus of saline at the same time point. A further control group was performed to identify any effects of DEX alone in this model, with LPS replaced by a 60 min infusion of saline. All groups were compared to saline/saline controls.

Parameter	Time point	Saline/Saline (Grp 1)	Saline/LPS (Grp 2)	DEX/Saline (Grp 3)	DEX/LPS (Grp 4)
Spleen DR	4 h post LPS	0.20 ± 0.03	0.40 ± 0.05 (a)	0.17 ± 0.01	0.21 ± 0.02 (b)
Liver DR	4 h post LPS	0.20 ± 0.04	0.41 ± 0.05 (a)	0.17 ± 0.004	0.20 ± 0.01 (b,c)
% Change WBC	End LPS (1 h)	-12 ± 11	-45 ± 3 (a)	-26 ± 8	-35 ± 10
(From time 0)	4 h post LPS	-1 ± 8	-41 ± 4 (a)	+19 ± 38	+210 ± 46 (a,b,c)
% Change Platelet Count	End LPS (1 h)	-17 ± 8	-8 ± 5	+2 ± 7	-16 ± 4
(From time 0)	4 h post LPS	-12 ± 5	-33 ± 4 (a)	+4 ± 5	-20 ± 7 (c)

Data are mean ± sem, n=6 per group. (a) = $p<0.05$ vs Grp 1; (b) = $p<0.05$ vs Grp 2; (c) = $p<0.05$ vs Grp 3; Mann-Whitney U-test.

These data show that LPS (Grp2) caused significant fibrin deposition in spleen and liver compared to saline (Grp 1). DEX (Grp 4) significantly alleviated this LPS-induced fibrin deposition. DEX alone had no significant effects compared to saline/saline controls on any parameter measured. DEX did not prevent the initial leukopenia characteristic of this model, but at the end of the experiment white blood cell count was elevated significantly in the DEX/LPS group (Grp 4) compared to all other study groups. Pretreatment with DEX had no effect on the thrombocytopenia induced by LPS (Grp 4 vs Grp 2). No significant effect of DEX on blood pressure was observed, however it should be noted that this is not a model of septic shock. These data indicate the potentially modulatory effects of agents such as DEX in DIC. However, since DEX may have a multitude of actions in such a complex syndrome, it is not possible to conclude from the present data exactly how DEX is exerting its' effects. It has been postulated that, in addition to an inhibitory effect on the induction of the Ca^{2+} independent NO synthase (Wright et al., 1992), DEX may inhibit the LPS-induced release of cytokines such as tumour necrosis factor which have been implicated in the evolution of DIC (Meir et al, 1990). These results suggest further studies with more selective interventions to elucidate the mechanism of action of DEX in this model, which may help to further the understanding of the interactions involved in the evolution of DIC.

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Meir, J.W., et al., (1990). Blood, 76 (10): 1933-1940.
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75P DIFFERENTIAL EFFECTS OF LIPOPOLYSACCHARIDE ON THE CAROTID HAEMODYNAMIC RESPONSES TO ACETYLCHOLINE AND METHOXAMINE IN CONSCIOUS LONG-EVANS RATS

J. Waller, S.M. Gardiner, and T. Bennett. Department of Physiology and Pharmacology, Queen's Medical Centre, Nottingham NG7 2UH.

We have previously described the renal, mesenteric and hindquarters haemodynamic responses to a chronic low dose infusion of lipopolysaccharide (LPS) (Gardiner *et al.*, 1992). In the present study, we used the same protocol to investigate effects of LPS on carotid haemodynamics, and on responses to acetylcholine (ACh) and methoxamine (ME).

Male Long Evans rats were anaesthetised (sodium methohexitone, 40-60 mg kg^{-1} i.p.) and had pulsed Doppler probes implanted around both common carotid arteries after the left external carotid artery had been ligated. Seven to 14 days later, animals were anaesthetised and had intravascular catheters implanted. At least 24h later changes in cardiovascular variables were measured during 3 min infusions 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}).

During saline infusion there were no marked changes in either baseline cardiovascular status or in the responses to ACh or ME. Table1 summarises the effects of LPS infusion on both resting cardiovascular variables and on the response to ACh infusion.

Table 1. Resting values (for heart rate (HR), mean arterial pressure (MAP), internal carotid vascular conductance (ICVC), and common carotid vascular conductance (CCVC)), 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=9).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)	ICVC ([kHz mm Hg^{-1}]10^3)	CCVC ([kHz mm Hg^{-1}]10^3)	ΔHR (beats)	ΔMAP (mm Hg min)	ΔICVC ([kHz mm Hg^{-1}]10^3)min)	ΔCCVC ([kHz mm Hg^{-1}]10^3)min)
Control	351 ± 17	105 ± 2	23 ± 2	41 ± 5	181 ± 25	-34 ± 10	19 ± 4	48±5
LPS 2h	376 ± 9	91 ± 3*	27 ± 2*	46 ± 6	96 ± 18*	-41 ± 10	42 ± 5 *	105 ± 13*
LPS 6h	368 ± 8	109 ± 4	17 ± 1*	28 ± 5*	92 ± 20*	-25 ± 9	16 ± 2	32±6*
LPS 24h	419 ± 20*	88 ± 3*	33 ± 3*	60 ± 6*	44 ± 13*	-66 ± 13*	42 ± 5*	95±13*

Two h after the onset of the infusion of LPS, there was a modest fall in MAP associated with a rise in ICVC; the carotid vasodilator responses to ACh were enhanced, whereas the pressor and vasoconstrictor responses to ME were significantly (P<0.05) reduced (ΔMAP, 97 ± 9 to 33 ± 5 mmHg min; ΔICVC, -26 ± 3 to -17 ± 3 ([kHz mm Hg^{-1}]10^3) min; ΔCCVC, -51 ± 11 to -18 ± 5 ([kHz mm Hg^{-1}]10^3) min). After 6h of LPS infusion, MAP was normal but there was vasoconstriction in internal and common carotid vascular beds; common carotid vasodilator responses to ACh and all responses to ME were significantly (P<0.05) reduced (ME: ΔMAP, 32 ± 6 mmHg min; ΔICVC, -11 ± 2 ([kHz mm Hg^{-1}]10^3) min; ΔCCVC, -12 ± 3 ([kHz mm Hg^{-1}]10^3) min). After 24h of LPS infusion there was hypotension associated with marked carotid vasodilation; ACh responses were again enhanced whereas responses to ME were still significantly (P<0.05) reduced (ME: ΔMAP, 20 ± 3 mmHg min; ΔICVC, -16 ± 2 ([kHz mm Hg^{-1}]10^3) min; ΔCCVC, -23 ± 3 ([kHz mm Hg^{-1}]10^3) min).

The changing pattern in resting values during LPS infusion, and the differential effects of LPS on responses to ACh and ME suggest that the results cannot simply be explained by a single phenomenon, e.g., the induction of nitric oxide synthase, but are more likely to involve the interplay of several mechanisms, for example prostanoid production, sympathoadrenal activation and vasopressin release.

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76P ANTIBODY ISOTYPE AFFECTS THE EFFICACY OF ANTI-TUMOUR NECROSIS FACTOR ANTIBODIES ON LPS-INDUCED SHOCK IN MICE

A.J. Suitters, M.W. Bodmer & R. Foulkes. Celltech Limited, 216 Bath Road, Slough, Berkshire. SL1 4EN.

The isotype (Fc region) of an antibody determines its ability to mediate certain effector functions such as complement fixation and Fc receptor binding. For murine antibodies this effector capability is mediated by the gamma 2a isotype, whereas the gamma 1 does not mediate these functions. It follows, therefore, that in certain disease conditions where monoclonal antibody (MAb) therapy is being considered, the choice of isotype may be important in determining the overall effectiveness of treatment. MAbs to TNF have previously been shown to protect against LPS-induced shock (Beutler, *et al.*, 1985; Sheehan *et al.*, 1989). Therefore in the present study the effect of different antibody isotypes on the efficacy of anti-tumour necrosis factor (TNF) monoclonal antibodies (MAb) with the same antigenic specificity was investigated using a lethal model of LPS-induced shock in mice. A series of hamster murine chimeric anti-TNF MAbs were made using the hamster anti-murine TNF MAb, TN3 1912, and replacing the hamster Fc region with either a murine gamma 1 (cTN3 1912 g1) or gamma 2a (cTN3 1912 g2a) Fc region.

Male Balb/c mice (16-20 gms) received lipopolysaccharide (LPS) at a dose of 50mg/kg i.v. Antibodies were given as a single pretreatment dose i.v. one hour prior to LPS challenge at doses of 30, 3 and 0.3mg/kg (n=10 per group). Saline controls were included. Animals were assessed 4 times daily and surviving animals killed at 72 hours. Statistical analysis of the Kaplan-Meier survival curves was performed using the Log Rank test and Wilcoxon test comparing antibody treated survival rates with the untreated controls.

There was 100% mortality in the untreated controls by 27 hours. cTN3 1912g 2a MAb at 30mg/kg gave minimal protection with 10% survival at 72 hours (p=<0.05). No protection was seen with 3mg/kg and 0.3mg/kg dose with 100% mortality by 25.5 and 21 hours respectively. Treatment with 30mg/kg cTN3 1912 g1 MAb increased survival to 90% by 72 hours (p=<0.004). There was prolongation of survival to 45 hours using the antibody at 3mg/kg (p=<0.05). No protection was seen with the 0.3mg/kg cTN3 1912 g1 with 100% mortality by 27 hours. A comparison of the survival probabilities of the two antibodies at 30mg/kg showed a significant difference (p=<0.004) between cTN3 1912 g1 and cTN3 1912 g2a.

In conclusion, anti-TNF antibodies prevented mortality following LPS challenge in a dose-dependent manner as shown by other workers (Beutler *et al.*, 1985; Sheehan *et al.*, 1989). It is clear that antibody isotype can substantially affect the efficacy of an antibody. Therefore studies comparing the activities of different isotypes are important in deciding the best strategy for immunotherapy in various human diseases.

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77P ANTI-TUMOUR NECROSIS FACTOR α MONOCLONAL ANTIBODIES (ANTI-TNF MAb) ARE THERAPEUTICALLY EFFECTIVE IN A MODEL OF COLONIC INFLAMMATION

P.S.Ward, S.R. Woodger, M.Bodmer & R.Foulkes, Celltech Ltd, 216 Bath Road, Slough, SL1 4EN.

Ulcerative colitis is a debillitating disease, of well described pathology, but unknown cause. TNFα has been proposed as a mediator of this inflammation. Mild irritant enemas act as experimental systems to study colonic inflammation (Cominelli *et al*, 1989). We describe here the results of an experiment to study the effect of a panel of anti-TNF MAb (TN3 19.12, hamster anti-murine, or chimeric murine anti-murine gamma1 (g1, non-complement fixing) or gamma2a (g2a, complement fixing, mediates Fc-effector function) isotypes) on such a system. Adult male Sprague-Dawley rats were divided into 5 groups (n=6-16): saline control, inflammation alone, or inflammation plus TN3, g1 or g2a respectively. Inflammation was induced, under halothane anaesthesia, by an acetic acid enema (1ml;2.5%) 10cm along the colon. After 2h, pre-formed immune complexes (BSA/anti-BSA) were given *i.v.*. Anti-TNF was given 24h before acetic acid (i.p;15mg.kg^{-1}), then 3x weekly. Rats were housed in metabolism cages, to collect faeces, and weighed regularly. Faecal % H_2O was measured by drying at 120°C, and total water excretion extrapolated from total faecal excretion. After 10 days the animals were killed and colonic tissue removed and fixed in formalin. Sections were cut and stained (Vital New Red), and counts of infiltrating eosinophils made along the base of the crypts. Data was analysed by ANOVA followed by Students t-test or Wilcoxon Sign Rank tests as appropriate.

	Saline	Inflammation	TN3 19.12	g1 isotype	g2a isotype
Weight diff.(g)	11.33± 3.21**	-18.87± 4.19**	-9.8± 4.51	-10.94± 4.22	-9.4± 4.38
Faecal water	2.9± 1.35	18.75± 2.39***	3.84± 4.29	3.69± 3.32	7.32± 4.45
Eosinophils	2.05± .17	9.17± 1.19***	7.52± .93**	5.04± .67$^{**, \$\$}$	5.15± .59$^{**, \$\$}$

Table 1. Data are mean ± s.e.mean. Weight diff. is at day 4. Faecal water is change from day 0, in g $H_2O.g^{-1}$ body weight.day^{-1}. Eosinophils are counts/ field of view. For weight change ** is p<0.01 vs. day 0. Elsewhere, *** p<0.001, ** p<0.01 vs. saline, $$ p<0.5 vs. inflammation.

Acute colonic inflammation was initiated by installation of acetic acid and pre-formed immune complexes. This was manifested as weight loss, diarrhoea and an influx of inflammatory leukocytes. The weight loss was maximal at day 4, and was mainly a result of the animals not eating. The weight loss in all three anti-TNF treated groups was not significant. Diarrhoea was noted in the inflammation-only group, but was completely attenuated by all three drugs. Eosinophils were present in all groups after acetic acid, but were reduced by treatment with the two chimaeric antibodies. Away from areas of necrosis, which were rare, neutrophils were rarely seen. In conclusion, this experiment shows that colonic inflammation results in functional and pathological changes, which are attenuated by administration of an anti-TNF MAb. This suggests that anti-TNF warrants further investigation as a potential therapy in IBD.

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78P NEURONAL CONTRIBUTION TO IMMEDIATE HYPERSENSITIVITY REACTIONS IN ISOLATED COLONIC MUCOSA

K.E. O'Malley, [1]P. Joyce & A.W. Baird, Departments of Pharmacology & Zoology[1], University College Dublin, Belfield, Dublin 4, Ireland.

Type 1 hypersensitivity reactions appear to contribute to host protection from enteric parasites (Wakelin, 1993). The effector system may involve IgE and mast cell mediated intestinal ion transport in parasitised animals during antigen challenge (O'Malley & Baird, 1993). The object of this study was to investigate possible involvement of intrinsic neurons within the lamina propria in antigen-evoked ion transport.

Male Wistar rats (150 - 200g) were actively infected with *Fasciola hepatica*. Detection of specific serum antibody (raised against crude, dialysed homogenate of mature *F. hepatica*) was used as a sign of successful infection. 3 weeks later, colonic mucosae, stripped of underlying smooth muscle,but with intact lamina propria, were mounted in Ussing chambers (window area = 0.63cm^2) and bathed in oxygenated Krebs-Henseleit solution at 37°C. Tissues were voltage clamped by continuous application of short circuit current (SCC). Antigen was prepared as a dialysed homogenate of adult fluke and expressed in terms of protein content. Drugs and antigen were added basolaterally to the serosal side bathing solution. Results are expressed as mean ± standard error of the mean.

Challenge of sensitised colon with antigen (25.0μg/ml; n = 17) caused a rapid-onset, transient increase in inward SCC (30.5±3.7μA). There was no response in non-sensitised tissues or with apical antigen challenge in sensitised mucosae. Under chloride-free conditions, the stimulating effect of antigen on SCC was reduced by 97.8±0.6% (n = 6; p < 0.005), thus confirming that antigen-evoked ion transport is accounted for by non-specific chloride secretion. Goat antibodies against rat IgE, produced qualitatively similar SCC responses in both normal (12.0±3.0μA; n = 5) and sensitised (10.6±1.5μA; n = 5) colonic epithelia presumably by provoking degranulation of mast cells within the lamina propria.

Tetrodotoxin (TTX; 1μM) reduced the SCC response to antigen (25.0μg/ml) by 61.7±15.0% (n = 4; p < 0.05), thus suggesting that enteric neurons of the lamina propria may amplify responses to antigen challenge. The SCC response to anti IgE (40μl) was also reduced by TTX (66.2±14.8%; n = 5; p < 0.005). Prostaglandin E_2 (10μM), a powerful stimulant of chloride secretion in this tissue (30.0±5.5μA; n = 5), was not significantly reduced by TTX (87.0±13.2% of control values). Capsaicin (100μM) stimulated SCC (19.7±2.1μA; n = 6) and also attenuated antigen-evoked SCC response by 75.1±5.8% (p < 0.05) without significantly affecting the SCC response to forskolin (10μM) which was 88.6±10.6% of control values (51.8±10.5μA; n = 6).

These results suggest that intrinsic neurons, strategically located in close proximity to mast cells in the lamina propria of the gut, may be involved in local intestinal anaphylactic reactions which contribute to host protection from parasite infection.

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79P HISTAMINE STIMULATION OF HUMAN COLONIC ION TRANSPORT IS MEDIATED VIA H_1 RECEPTORS

S.J.Keely, W.A. Stack[1], D.P. O' Donoghue[1] and A.W. Baird, Department of Pharmacology, University College Dublin and [1]Gastroenterology and Liver Unit, St. Vincent's Hospital, Dublin 4, Ireland.

Chloride secretion in response to histamine has been demonstrated across guinea pig colonic epithelia. This action is a consequence of both direct and indirect effects of histamine on epithelial cells since activation of intrinsic nerves and endogenous eicosanoid synthesis contribute to the secretion (Wang *et al.*, 1990). In this study we have used human isolated colon to identify receptor subtype(s) involved in the response to histamine, to identify the charge carrying ion(s) involved and to determine if histamine has indirect actions via activation of enteric neurons or by stimulation of eicosanoid synthesis. Segments of histologically normal colon obtained during surgical resection were stripped of underlying smooth muscle, mounted in Ussing chambers and voltage clamped by continuous application of short circuit current (SCC). Results are expressed as mean ± s.e. mean; n=5 throughout. Statistical analysis was carried out by analysis of variance or by student's t-tests. Paired preparations were obtained from each surgical specimen.

i) Histamine added to the basolateral side stimulated SCC (EC_{50} = 0.14±0.03mM). Apical side application of histamine did not alter SCC. The H_2- and H_3-selective agonists dimaprit and s(+) α-methyl histamine were without effect at concentrations up to 1mM. Responses to histamine were abolished by the H_1 receptor antagonist, mepyramine (100μM; $p<0.005$) but were unaffected by cimetidine (100μM) or thioperamide (100μM) which are H_2- and H_3-selective antagonists. Mepyramine did not alter the SCC response to carbachol (100μM).

ii) Electrogenic ion transport responses to histamine were virtually abolished by bumetanide (100μM; $p<0.01$) but were not attenuated by amiloride (10μM) or acetazolamide (1mM). These results suggest that chloride secretion is the predominant ion transport response to histamine in human colon.

iii) SCC responses to histamine were not altered by tetrodotoxin (1μM), by the cyclooxygenase inhibitor, piroxicam (10μM) or by the lipoxygenase inhibitor, nordihydrogauertic acid (NDGA). However, a combination of piroxicam and NDGA significantly attenuated the action of histamine (30-100μM; $p<0.05$). SCC responses to carbachol (100μM) were 46.5±8.7μA in the presence of piroxicam and NDGA which were not different from results obtained in matched control preparations (43.4±17.9 μA).

These results show that histamine stimulates chloride secretion across human colonic epithelium. The action is mediated via H_1-receptors and does not appear to be mediated by tetrodotoxin-sensitive neurons. However, full expression of the pro-secretory action of histamine may involve products of arachidonic acid metabolism. Inhibitory effects were observed only in the presence of both blockers as inhibition of either pathway alone may result in a compensatory stimulation of the alternative pathway. Reciprocal interactions between the lipoxygenase and cyclooxygenase pathways have been previously demonstrated in human colon (Wardle *et al.*,1993).

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80P AGONIST-INDUCED DESENSITITATION OF THE HISTAMINE H_1-RECEPTOR IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS IS INDEPENDENT OF PROTEIN KINASE C

G. McCreath, I.P. Hall & S.J. Hill[1] , Division of Therapeutics and [1]Department of Physiology & Pharmacology, University Hospital of Nottingham, Nottingham NG7 2UH

We have previously reported the desensitisation of the histamine H_1-receptor-mediated inositol phospholipid hydrolysis in cultured human umbilical vein endothelial cells (HUVEC)(McCreath et al.,1992). Desensitisation is the result of prior exposure of cells to histamine and is mimicked by protein kinase C (PKC) activation (McCreath et al.,1992). In the present study we have examined the effect of the PKC inhibitor, staurosporine, on histamine-induced H_1-receptor desensitisation in HUVEC.

Endothelial cells were obtained from fresh umbilical veins following the procedure of Jaffe et al.(1973) and were cultured at 37°C in humidified air/CO_2 (95;5) until reaching confluence. Monolayer cell cultures were loaded in 24-well cluster dishes with ^{3}H-myo-inositol (1μCi/well) for 24 hrs previous to agonist stimulation. Total ^{3}H-inositol phosphates were measured as previously described (Hawley et al,1992).

Histamine (0.1mM) produced a 12.7 ± 0.2 fold increase in ^{3}H-inositol phosphate accumulation (n=65). Prior treatment of HUVEC with histamine (100nM to 0.1mM, 2 hour period) produced a marked decrease (IC_{50}=0.97 ± 0.2μM;n=10) in the subsequent ^{3}H-inositol phosphate response to 0.1mM histamine (45 min. stimulation). There was no significant change in either the histamine IC_{50} (1.7 ± 0.3μM and 1.7 ± 0.2μM) or the maximal inhibition (59 ± 2.8% and 50 ± 3%;n=4) induced by desensitisation following vehicle or staurosporine (100nM) treatment respectively. In marked contrast, staurosporine (100nM) was able to reverse the inhibitory effect of phorbol dibutyrate (PdBu)(1μM, 2hr) on histamine (0.1mM) H_1-mediated phosphoinositide hydrolysis (max. inhibition was 64 ± 1.8% in the absence of staurosporine (100nM) whereas PdBu (1μM) pretreatment, following exposure to staurosporine (100nM), produced enhancement of response by 26 ± 0.7%). Histamine (0.1mM) pretreatment did not significantly reduce the response to stimulation by sodium fluoride (NaF)(20mM;n=5) or thrombin (1U/ml;n=4).

In summary, these data suggest that histamine pretreatment may produce a homologous desensitisation of the H_1-receptor in HUVEC and furthermore they do not provide any evidence for a major role of PKC in this desensitisation process.

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81P REGIONAL AUTORADIOGRAPHIC LOCALISATION OF NITRIC OXIDE SYNTHASE IN THE RAT BRAIN USING [^{3}H]NITROARGININE

EJ Kidd, AD Michel & PPA Humphrey.
Glaxo Institute of Applied Pharmacology, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QJ.

Nitric oxide synthase (NOS) is the enzyme responsible for the production of nitric oxide, a putative neurotransmitter. The distribution of this enzyme in rat brain (Vincent & Kamura, 1992) and spinal cord (Valtschanoff et al., 1992) neurones has previously been studied using the NADPH-diaphorase technique. However, this method may not be specific for NOS and therefore a more selective tool would be valuable. Nitroarginine is an inhibitor of NOS which has recently become available in a tritiated form. We have shown that the binding of this radioligand to the soluble enzyme can be prevented by L-arginine (Michel et al., 1993) and have now examined the regional distribution of its binding in the rat central nervous system (CNS) using autoradiography.

Frozen sections (20 μm) of unfixed brain and spinal cord, on coated slides, were preincubated in 50 mM Tris.HCl, 3 mM $CaCl_2$ and 0.025% Triton X100 buffer, pH 7.4 for 30 min at 25°C, then incubated for 60 min at 4°C with ~7 nM [^{3}H]nitroarginine (Michel et al., 1993) with or without 1 μM nitroarginine for non-specific binding, washed for 3 x 10 min and dipped for 10 sec in ice-cold distilled water. The dried slides were apposed to X-ray film for 3 months and the images were quantified using suitable standards.

The highest levels of specific binding (grey values, mean ± s. e. mean, n ≥ 19) were found in the tenia tecta (0.155 ± 0.009), throughout the amygdaloid complex, in particular in the anterior (0.107 ± 0.007) and medial (0.125 ± 0.006) nuclei, the amygdalopiriform transition (0.134 ± 0.005) and in the anterior cerebellum (0.087 ± 0.002). There was also binding in the thalamic bed nucleus of the stria terminalis (0.067 ± 0.005), the dentate gyrus (0.033 ± 0.001), the ventral hippocampal CA3 area (0.051 ± 0.002), layers 1 and 2 of the frontal cortex (0.064 ± 0.003) and the superior gray layer of the superior colliculus (0.053 ± 0.002). The paraventricular nucleus of the hypothalamus and the superficial layers of the dorsal horn of the thoracic and lumbar spinal cord were also labelled but this was not quantifiable at this time point. Binding in the striatum and the rest of the cortex was diffuse and therefore not quantified. There appeared to be few or no specific binding sites in the pons and medulla.

The regional distribution of binding sites for [^{3}H]nitroarginine in the rat CNS was, in general, similar to that seen with the diaphorase method. There were, however, differences in the cortex and the hindbrain which may be due to the existence of more than one isoform of NOS in the CNS. Thus, [^{3}H]nitroarginine appears to be a very useful ligand for studying the distribution of NOS in the CNS and may also be capable of distinguishing between potential isoforms. More studies are planned using dual labelling techniques to determine whether [^{3}H]nitroarginine binds to cells, labelled either with the diaphorase method or a specific antibody to the cloned brain isoform of NOS.

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82P HIGH-AFFINITY UPTAKE OF L-ARGININE BY CEREBELLAR AND CORTICAL SYNAPTOSOMES

Christian R. Aldridge & Keith J. Collard, Department of Physiology, University of Wales College of Cardiff, P.O. Box 902, Cardiff CF1 1SS.

There is good evidence that nitric oxide (NO) may have transmitter-like functions in the peripheral nervous system (Belvisi et al. 1992), but little is known about such a role in the CNS. In order to function in a transmitter-like manner, NO must be formed locally within nerve endings. NO synthase is present in synaptosomal fractions (Knowles et al. 1990), but the precursor amino acid is formed mainly in glial cells (Aoki et al. 1991). Consequently, a transcellular transport of L-arginine has been suggested to operate in which L-arginine released by glial cells is taken up by neurones and used for the production of NO (Hansel et al. 1992).

In this preliminary study, we have re-examined the transport of L-arginine in synaptosomes prepared from rat cerebellum and cortex. Standard procedures routinely used in our laboratory to study synaptosomal amino acid transport were adapted for this study (Wilkinson & Collard 1984).

The kinetics of the uptake of L-arginine at 37°C revealed that uptake could be resolved into a single saturable carrier-mediated process with minimal diffusion. Linear transformation of the data provided values for Km and Vmax as follows: Cortical synaptosomes, Km 26.3 uM, Vmax 357.1 pmol/mg protein/min; cerebellar synaptosomes, Km 45.45 uM, Vmax 512.8 pmol/mg protein/min. Using an incubation concentration of 10 uM, L-arginine uptake (pmol/mg protein/min) in Na^+-rich Krebs in cortical and cerebellar synaptosomes was 94.02 ± 3.9 and 94.13 ± 9.76 respectively. In the absence of extracellular Na^+ it was 225.21 ± 11.06 and 234.33 ± 15.7 (n=5). The difference between uptake in Na^+-rich and Na^+-free conditions was significant ($P<0.001$ Student's t test).

The results of this study have revealed the presence of a high-affinity L-arginine uptake system which differs kinetically and in its Na^+-dependency from the low-affinity carrier previously described (Snyder et al. 1973). The relationship between this carrier and the production of NO in nerve endings has yet to be investigated. However, the high-affinity nature of the carrier and its inhibition by Na^+ suggests that it is a carrier which may be involved in the production of neurally active material and which may be regulated by changes in ion distribution across the plasma membrane.

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83P BEHAVIOURAL RECOVERY FOLLOWING INTRASTRIATAL LESIONS WITH QUINOLINIC ACID IS NOT RELATED TO SURVIVAL OF NITRIC OXIDE SYNTHASE-POSITIVE NEURONES

L. M. Tucker, P. C. Emson[1] & A. J. Morton (introduced by J.M.Edwardson), Department of Pharmacology, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QJ, UK and [1]Cambridge Research Station, Babraham, Cambridge CB2 4AT, UK.

Intrastriatal injections of quinolinic acid (QA) are said to induce pathology which is similar to that of Huntington's disease, including the relative sparing of neurones containing NADPH-diaphorase (Beal et al., 1986). However, using high QA concentrations we find extensive loss of these neurones. With the discovery that NADPH-diaphorase is the neuronal form of nitric oxide synthase (NOS; Hope et al., 1991) there has been speculation about the role which neurones containing this enzyme may play in the pathogenesis of excitotoxic neuronal damage.

We examined the effect of unilateral, intrastriatal injections of QA (10, 30, 50 and 100 nmol) on the survival of NOS-positive neurones in rat striatum for periods up to three months. We used methamphetamine-induced rotation as a measure of the striatal damage induced by QA, and looked to see if there was a correlation between the level of drug-induced rotation and the sparing of NOS-containing neurones. Methamphetamine (5 mg/kg i.p.) was administered to rats lesioned with QA up to 3 months previously. Rotations were counted over the next 60 minutes in an automated rotometer. After testing, the rats were perfusion-fixed with 2% paraformaldehyde in 0.1M PBS and cryostat sections (30μm) of brain were cut and stained immunocytochemically for NOS using a rabbit antiserum (gift of Dr. A. Davenport). NOS-positive cells in the lesioned and contralateral unlesioned striata were counted in 6 serial sections (150μm apart) from each rat (3 rats per group).

Rotation (mean turns/hour ± SEM for each group) increased over the first two weeks following lesioning with 50 nmol QA (from 24±80 at 1 day to 531±170 at 9 days) suggesting that striatal damage was progressive over this period. The increase seen after 100 nmol QA (from 266±202 at 1 day to 653±153 at 9 days) was not significant. (Vehicle-lesioned control rotation was 23±10 at 9 days). By 23 days post-lesion, rotation was significantly reduced in rats with 50 nmol, but not 100 nmol QA lesions (control, 31±49; 50 nmol QA, 124±161; 100 nmol QA, 523±150). After 30 nmol QA, there was some rotation at two weeks (53±18 turns/hour) but by three months rotation was no longer seen. Rats with 10 nmol QA lesions did not rotate. The size of the lesions as determined by the extent of reactive gliosis was directly proportional to the amount of QA injected. There was extensive loss of NOS-positive neurones from all lesions but not from regions immediately beyond the lesion. This loss was apparent on day 1 after lesioning and persisted for the duration of the study. Thus, although there was a positive correlation between the rotational deficits seen with increasing doses of QA and the loss of NOS-positive neurones, the behavioural improvements seen in the 30 and 50 nmol QA-lesioned groups were not due to selective sparing or recovery of NOS-positive neurones.

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84P POTENTIATION AND INHIBITION OF ENDOTOXIN-INDUCED VASCULAR INJURY IN RAT INTESTINE BY NITRIC OXIDE SYNTHASE INHIBITORS

F. Laszlo, N.K. Boughton-Smith, B.J.R. Whittle and S. Moncada. Wellcome Research Laboratories, Beckenham, Kent BR3 3BS.

Nitric oxide (NO), formed by a constitutive enzyme, plays a role in the modulation of intestinal vascular integrity. Thus, inhibition of NO synthase augments the plasma leakage in rat gastro-intestinal tissue seen 15 min following high doses of endotoxin, and provokes acute vascular injury in the cat intestine (Hutcheson et al, 1990; Kubes & Granger, 1992). By contrast, induction of a NO synthase by lower doses of endotoxin (Salter et al, 1991) over 3-5h is associated with vascular injury in the rat intestine (Boughton-Smith et al, 1992). The effects of administration of the NO synthase inhibitors, N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA) during the 5h period following challenge with these lower doses of endotoxin, on intestinal vascular permeability to albumin have now been evaluated.

Under halothane anaesthesia, E. coli lipopolysaccharide (LPS; 3mgkg^{-1} i.v.) was administered to rats (male, 225-275g). [^{125}I] human serum albumin (2μCi kg^{-1} i.v.) was administered for the subsequent determination of vascular albumin leakage as an index of endothelial injury. Five hours after LPS administration, there was a significant ($P<0.05$) increase in albumin leakage into the ileal and colonic tissue (Δ87±16 and 55±10μl g^{-1} tissue, respectively, mean±s.e.mean; n=22 for each). Concurrent administration of L-NAME (1-5mgkg^{-1} s.c.) produced a dose-dependent aggravation of LPS-provoked albumin leakage observed after 5h. Thus, with L-NAME (5mgkg^{-1} s.c.), albumin leakage was significantly ($P<0.05$) elevated to Δ670±119 and 234±66μl g^{-1} tissue respectively (n=8 and 10). It is known however, that activity of the calcium-independent inducible enzyme is only detected in these intestinal tissues from 3h after LPS challenge (Boughton-Smith et al, 1992). Thus, administration of L-NAME (1-5mgkg^{-1} s.c.) 3h after LPS, caused a dose-dependent reduction in the vascular injury in both ileum and colon, determined 2h later, with albumin leakage being abolished with the higher dose (n=8, $P<0.01$ for both). Likewise, administration of L-NMMA (12.5-50mgkg^{-1} s.c.) 3h after LPS, dose-dependently reduced the albumin leakage in both ileum and colon observed 2h later, being abolished by the higher dose (n=8; $P<0.001$ for both). Neither L-NAME (5mgkg^{-1}) or L-NMMA (50mgkg^{-1}) affected albumin leakage under control conditions, 5h after administration in either ileum or colon (n=8 for each).

These findings confirm that initial suppression of the constitutive NO synthase following challenge with LPS aggrevates the subsequent vascular injury in the ileum and colon. By contrast, administration of NO synthase inhibitors, at a time of detectable expression of the inducible NO synthase, protects against the subsequent vascular damage. These latter actions of these agents may reflect local constrictor effects following NO synthase inhibition in the intestinal microvasculature limiting the plasma leakage, or the inhibition of the excessive local generation of cytotoxic NO or its metabolites that injure the intestinal mucosa.

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85P OXIDISED LOW-DENSITY LIPOPROTEINS INHIBIT ENDOTHELIUM-DEPENDENT RELAXATIONS OF RABBIT CORONARY ARTERIES

C. Buckley, *S. Bund, *F. McTaggart, K. R. Bruckdorfer, M. Jacobs & *A. Oldham. Department of Pharmacology, Royal Free Hospital School of Medicine, London, NW3 2PF & *Cardiovascular Department, ZENECA Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, SK10 4TG.

Endothelium-dependent relaxations mediated by nitric oxide are impaired in arteries from atherosclerotic and hypercholesterolaemic animals This dysfunction has been linked to the presence of oxidised low-density lipoproteins (OXLDL) in the vessel wall. LDL oxidised with Cu^{2+} has been shown to inhibit endothelium-dependent relaxations in isolated rabbit aorta (Jacobs et al. 1990) and porcine coronary arteries (Tanner et al. 1991). In the present study the effects of native and oxidised LDL on endothelium-dependent responses of rabbit coronary arteries have been investigated.

LDL was prepared from fresh human plasma by discontinuous gradient ultracentrifugation and oxidised by incubation with Cu^{2+} (1nmole mg^{-1} LDL) for 24 hours at room temperature (Jacobs et al. 1990). Segments of the left circumflex coronary artery were dissected from the hearts of New Zealand White rabbits and mounted in a double myograph system maintained at 37°C and bubbled with 95% O_2, 5% CO_2. In each experiment the responses of segments from both large arteries of diameter 828.9 ± 19.2μm (n = 37) and small arteries of diameter 288.4 ± 12.7μm (n = 29) from the same animal were compared. After equilibration, vessels were constricted with either $PGF_{2\alpha}$ (5-8 x10^{-6}M) or 30mM K^+ and relaxed with cumulative concentrations of acetylcholine (ACh) (10^{-9}-10^{-5}M) or sodium nitroprusside (SNP) (10^{-8} -10^{-4}M). After a 30 minute washout period, tissues were exposed to LDL or OXLDL (0.5 or 1 mg protein ml^{-1}) for 30 mins before the constriction-relaxation cycle was repeated. A third relaxation curve to ACh was carried out after a further washout period to examine the reversibility of the effects of the lipoproteins.

Oxidised LDL reversibly inhibited ACh induced relaxations of $PGF_{2\alpha}$ constricted tissues. This is shown by a significant rightward shift in the ACh dose response curve in the presence of the lipoproteins. Incubation with 0.5mg ml^{-1} OXLDL resulted in an increase in EC_{50} values from 50 ± 10 nM to 122 ± 14 nM in large vessels and 91 ± 11 nM to 200 ± 29 nM in small vessels. The sensitivity to ACh was restored on washout of the lipoproteins. OXLDL did not have any effect on the maximum relaxation to ACh which was 93 ± 3% in control vessels and 92 ± 3% during exposure to OXLDL. The decrease in sensitivity was not significantly increased when vessels were exposed to 1mg ml^{-1} OXLDL. In vessels preconstricted with 30mM K^+, incubation with OXLDL also caused a reversible decrease in sensitivity to ACh. Incubation with 0.5mg ml^{-1} OXLDL significantly increased the EC_{50} values for ACh from 45 ± 11 nM to 114 ± 19 nM in large vessels and 79 ± 11 nM to 178 ± 21 nM in small vessels. In contrast to the effect observed in $PGF_{2\alpha}$ preconstricted vessels, there was also a significant decrease in the maximum level of relaxation in the presence of OXLDL. The maximum relaxation in control vessels was 56 ± 11% in large vessels and 47 ± 10% in small vessels. In the presence of 0.5mg ml^{-1} OXLDL this was reduced to 42 ± 7% and 33 ± 6%, on incubation with 1mg ml^{-1} OXLDL the maximum relaxation was further reduced to 32 ± 8% and 19 ± 7% in large and small vessels respectively. After the OXLDL was washed out the relaxations were restored. Native LDL (1 mg ml^{-1}) had no effect on endothelium-dependent responses of either large or small coronary vessels preconstricted with $PGF_{2\alpha}$. OXLDL (1mg ml^{-1}) had no effect on endothelium-independent relaxations evoked by SNP in either large or small vessels preconstricted with $PGF_{2\alpha}$.

In conclusion, this study demonstrates that oxidised but not native LDL causes a reversible inhibition of endothelium-dependent relaxations evoked by ACh in isolated rabbit coronary arteries. Furthermore, the extent of the inhibitory effect was similar in both large and small coronary vessels. These results provide further evidence that the presence of OXLDL in the vessel wall may contribute to the impairment of vascular function in the microcirculation observed in atherosclerosis and hypercholesterolaemia.

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86P THE ROLE OF NITRIC OXIDE IN ACETYLCHOLINE-EVOKED DILATATION OF THE RAT ISOLATED MESENTERIC BED

S. Parsons, C.J. Garland & F. Plane, Department of Physiology & Pharmacology, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU.

Acetylcholine (ACh)-evoked dilatation of the rat perfused mesenteric bed is inhibited by the NO synthase inhibitors L-N^G-nitroarginine (L-NOARG) and L-N^G-nitroarginine methyl ester (L-NAME; Moore *et al.*, 1990). However, in isolated segments of third order branch arteries from this vascular bed, ACh-evoked relaxations are resistant to blockade by L-NOARG, suggesting the release of a factor distinct from NO (Garland & McPherson, 1992).

In this study, the role of NO in the endothelium-dependent responses of the rat isolated perfused mesenteric bed to ACh and the calcium ionophore A23187 were further investigated. Isolated mesenteric preparations were perfused with warmed, oxygenated Krebs' buffer via a cannula inserted into the superior mesenteric artery. Bolus injections of ACh (0.02-2000 nmol) and the calcium ionophore A23187 (0.002-20 nmol) evoked dose-dependent dilatation of the mesenteric bed pre-constricted with noradrenaline (NA;10 μM). The maximal responses to acetylcholine and A23187 were 77.2 ± 7.6 % (n=9) and 65.4 ± 8 % (n=4) relaxation respectively. Infusion of L-NAME (100 μM for 30 mins) had no effect on basal perfusion pressure but depressed ACh-evoked dilatation of NA constricted tissues. The response to the lowest dose of ACh (0.2 nmol) was inhibited by 70 ± 4 % (n=5) and the maximal response (2000 nmol) was reduced by 35.7 ± 4 % (n=5). Similar results were obtained with L-NOARG. Perfusion of tissues with high potassium Krebs' buffer (25 mM KCl) prior to constriction with NA reduced both the amplitude and the duration of ACh-evoked dilatation. The response to the lowest dose of ACh (0.2 nmol) was reduced by only 7.6 ± 3 % (n=5) while the maximal response was inhibited by 72 ± 9 % (n=5). Exposure of tissues to both high potassium Krebs' buffer and L-NAME together, abolished responses to the lower doses of ACh and reduced the maximal response by 86.8 ± 7 % (n=4).

In contrast, A23187-evoked dilatation of NA-constricted tissues was unaltered following exposure to L-NAME. However, in the presence of 25 mM potassium, the response to the lowest dose of A23187 (0.002 nmol) was abolished and the maximal response (20 nmol) was inhibited by 80 ± 10 % (n=4). Indomethacin (10 μM) was without effect on responses to either ACh or A23187. These results indicate that ACh-evoked dilatation of the rat mesenteric bed has both NO synthase-dependent and -independent components, the contribution of each varying with the concentration of the agonist. In contrast, A23187-evoked dilatation is mediated by an NO synthase-independent mechanism.

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87P L-CANAVANINE INHIBITS THE RECYCLING OF L-CITRULLINE TO L-ARGININE IN THE RAT ANOCOCCYGEUS MUSCLE

Emma J. Mickley, Timothy D. Warner and John R. Vane. *The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ, U.K.*

L-N^G-nitro-arginine (Gibson et al., 1990) inhibits the nitric oxide-(NO)-dependent relaxations of the anococcygeus caused by activation of non-adrenergic non-cholinergic nerves (Brave et al., 1993). As L-citrulline reverses this inhibition (Gibson et al., 1990) the anococcygeus must convert L-citrulline to L-arginine, for the NO synthase present is like that in brain (Mitchell et al., 1991) for which L-citrulline is not substrate. Here we have characterised this further, for recycling of L-citrulline to L-arginine could be important in the regulation of NO synthase activity.

Male Wistar rats (200-350 g) were killed with pentobarbitone sodium (120 mg/kg), the anococcygeus muscles removed, and suspended in organ baths in warmed (37°C) and gassed (95%O_2:5%CO_2) Krebs' buffer containing phentolamine (10^{-6} M). During equilibration (45 min) tissues were exposed to guanethidine (3×10^{-5} M) for 10 min. The muscles were contracted with carbachol (5×10^{-5} M) and field stimulation (FS; 0.3 mA, 10 Hz, 0.1 ms, 10s every 100s) applied to cause reproducible relaxations. After washing, tissues were exposed to L-N^G-nitro-arginine methyl ester (L-NAME, 10^{-4} M) and/or L-canavanine (10^{-3} M), and/or continual FS (0.3 mA, 10 Hz, 0.1 ms). Carbachol was then re-applied and responses to FS recorded with and without the addition of L-arginine (10^{-3} M) or L-citrulline (10^{-3} M).

L-arginine or L-citrulline reversed equally the inhibition of relaxations induced by L-NAME, but only L-arginine reversed the inhibitory effects of L-NAME + L-canavanine (Table 1). Exposure to L-canavanine did not inhibit relaxations to FS (n=10), but did when coupled with prolonged FS, an effect which was reversed by L-arginine but not L-citrulline.

Treatment	control	+ L-arginine (10^{-3} M)	+ L-citrulline (10^{-3} M)
L-NAME (10^{-4} M)	5.7±1.1%	35.1±7.0%*	41.2±5.2%*
L-NAME (10^{-4} M) + L-canavanine (10^{-3} M)	2.6±1.1%	37.9±8.7%*	9.9±2.7%*
continual FS (90 min)	27.5±3.5%	35.0±4.2%	28.4±5.3%
continual FS (90 min) + L-canavanine (10^{-3} M)	8.1±1.8%	31.4±5.1%*	10.8±2.3%

Table 1. Relaxations induced by electrical FS. (*$p<0.05$, w.r.t. control, student's *t*-test).

We conclude that in the anococcygeus muscle L-citrulline is converted to L-arginine by an enzyme that is inhibited by L-canavanine. Thus, L-canavanine prevents the reversal of L-NAME by L-citrulline and the maintenance of NO production during periods of prolonged stimulation.

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88P EFFECT OF D- AND L-ARGININE ON CONTRACTILE RESPONSES TO HISTAMINE AND BRADYKININ IN GUINEA-PIG ILEUM *IN VITRO*

A. M. Khawaja, A. C. Perez, W. Paul, C. P. Page & M. Schachter, Dept. of Pharmacology, King's College, London SW3 6LX

In studies of the role of the L-arginine (arg) - nitric oxide (NO) synthase pathway in mediator-induced cutaneous vascular permeability responses in the guinea-pig, co-injection (0.1ml site^{-1}) of L-arg (10μmol site^{-1}) enhanced plasma protein extravasation (PPE) induced by bradykinin (BK) whereas PPE induced by histamine (HA) was significantly reduced (Paul *et al.*, 1992). D-arg (10μmol site^{-1}) also had a significant inhibitory effect on HA-induced PPE whilst L-lysine (L-lys; 10μmol site^{-1}) had no effect on responses to BK or HA (Paul et al., 1992). The paradoxical effects of arg on responses to BK and HA could be explained if arg had anti-histamine activity at the concentration used in skin. We have now compared the effects of D- and L-arg and L-lys, at molar concentrations equivalent to those injected intradermally, on contractile responses to HA and BK in guinea-pig ileum (GPI) *in vitro*. GPI was suspended in gassed (95% O_2; 5% CO_2) Krebs solution at 34°C under 1 g tension for isometric recording. Dose-response (d/r) curves were constructed in Krebs with or without D-arg, L-arg or L-lys, all 100mM of the hydrochloride salt. Any one piece of ileum was exposed to only one amino acid. Responses were expressed as a % of the response to supramaximal effective concentrations of HA (25μM) or BK (1μM). EC_{50} values were calculated and expressed as the geometric mean (95% confidence limits) for n=4-6 replicates.

L-arg increased significantly ($p<0.05$) the EC_{50} for HA to 68.3 (8.3-563.2)μM from a control value of 0.4(0.1-1.5)μM. There was no significant change in the maximal response. In contrast, L-arg had no significant effect on responses to BK (EC_{50} values being 32.4(16.7-62.5)nM and 53.7(37.2-77.4)nM in the absence and presence of L-arg). D-arg increased the EC_{50} for HA from 113(60-211)nM to 3800(2000-7400)nM but also depressed the maximum response. L-lys had no significant effect on responses to HA (EC_{50} being 188(63-560)nM compared with 71(12-426)nM in controls).

These *in vitro* data on GPI confirm an old observation that arg inhibits HA responses in vitro (Frey *et al.*, 1950) and provide a plausible explanation for the discrepant effects of arg on BK- and HA-induced PPE. Whether the high concentration of arg used in the PPE and GPI experiments competes with HA for binding to HA receptors in a manner analogous to competition between alkyl esters of L-arg and muscarinic agonists (Buxton *et al.*, 1993) remains speculative. However, these observations indicate that caution is necessary when very high concentrations of arg are employed for assessing the role of NO in modulating tissue responses.

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89P GUANETHIDINE INHIBITS ENDOTHELIUM-DEPENDENT RELAXATION IN RABBIT AORTA

M.K. Dhiman, D. Lang, J.A. Smith* & M.J. Lewis, Department of Pharmacology &Therapeutics, and *Cardiology, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN

In this study we have compared the effects of the adrenergic neurone blocker guanethidine and the local anaesthetic agent lignocaine on acetylcholine(ACh)-induced endothelium-dependent relaxation of rabbit aorta and on the activity of constitutive NO synthase isolated from rabbit cerebellum.

The thoracic aorta of male New Zealand White rabbits (2-2.5g) was removed into Krebs buffer containing 0.01mM indomethacin and gassed with 95% CO_2/ 5% O_2 at 37°C. The cleaned aorta was cut into 2-3mm wide endothelium-intact rings and mounted in 7ml tissue baths for isometric tension recording. A resting tension of 2g was set. The cerebellum was removed and homogenised in ice cold buffer and prepared for NO synthase analysis as previously described (Salter *et al.*, 1991). All data are expressed as mean±s.e.mean (n≥4) and compared by fitting concentration-response curves using a least squares minimization procedure. For the mechanical studies tissues were preconstricted with phenylephrine (PE) to 80-90% of maximum. In control preparations PE, 10^{-6}M gave constrictions of 6±0.22g (n=8). In the presence of guanethidine or lignocaine higher concentrations of PE (up to 10^{-5}M) were required to produce a similar level of contraction eg 5.9±0.27g, n=8, in the presence of 10^{-3}M guanethidine and 5.8±0.36g, n=4 in the presence of 10^{-3}M lignocaine. Acetylcholine (10^{-8} to $3x10^{-6}$M) induced endothelium-dependent relaxations in PE-constricted rings reaching a maximum of 73.99±1.3% at $3x10^{-6}$M. This relaxation was significantly inhibited in a concentration-dependent manner by prior incubation for 10min with guanethidine(10^{-3} to 10^{-6}M). Relaxation in the presence of 10^{-3}M guanethidine was 39.2±4.55% ($p<0.01$). This relaxation was further inhibited in the presence of excess ($2x10^{-3}$M) L- (3.4±1.9%, $p<0.01$), and to a lesser extent D-arginine (25.7±5.56%, $p<0.05$)). ACh-induced relaxation was also significantly inhibited in the presence of 10^{-3}M lignocaine (61.62±1.00%, n=4, $p<0.05$). Both guanethidine and lignocaine produced a small rightward shift ($p<0.01$) in the concentration-response curve to sodium nitroprusside, but did not reduce the maximum reponse. Control Ca^{2+}-dependent NO synthase activity in the cerebellum preparation was 187.00±13.00 fmol of citrulline min^{-1} μg $protein^{-1}$. Neither guanethidine nor lignocaine (10^{-3} to 10^{-6}M) inhibited this activity at any of the concentrations studied. A small, but significant ($p<0.05$) increase in activity (260±23 fmol citrulline min^{-1} μg $protein^{-1}$) was seen in the presence of 10^{-6}M lignocaine.

The data presented show that guanethidine, at all concentrations studied, inhibited ACh-induced relaxation. This inhibition was potentiated in the presence of excess L- and to a lesser extent D- arginine. Lignocaine (10^{-3}M) similarly inhibited ACh-induced relaxation. Neither guanethidine nor lignocaine inhibited NO synthase activity. These agents therefore inhibit endothelium-dependent relaxation via a mechanism that does not involve NO synthase.

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90P BEHAVIOURAL EFFECTS OF APOMORPHINE IN FEMALE MICE

M.G. Cutler, Department of Biological Sciences, Glasgow Caledonian University, City Campus, Glasgow, G4 OBA.

The mixed D1/D2 dopamine receptor agonist, apomorphine, when given within a dose range which activates autoreceptors in the mesocortical and nigrostriatal dopamine system, has been shown to induce two distinct effects on the behaviour of male mice (Dixon et al. 1990). It increased defensive behaviour as well as suppressing locomotor activity. The present studies examine effects of apomorphine, given at similar dose levels, on the behaviour of female mice, an effect which has not previously been studied. Ethopharmacological methods were used to quantify effects on behaviour occurring during encounters of the females with unfamiliar male partners. This paradigm provides a sensitive measure of drug-induced changes to defensive behaviour and social investigation (Cutler, 1991).

Apomorphine hydrochloride, dissolved in physiological saline containing 0.1% ascorbic acid, was given to adult female CD1 mice at three dose levels (0.15 [LD], 0.30 [MD] and 0.60 [HD] mg/kg, i.p.; n=15 in each group). Control females (n=18) received physiological saline i.p. Behaviour shown by each mouse during social interactions was recorded for a 5 min. period at 30 min after injection, using the methods described by Dixon et al. (1990). All encounters were with an untreated male in an illuminated unfamiliar cage.

Apomorphine dose-dependently increased the duration of immobility in females (Control 0.2±0.1; Apomorphine; LD 2.0±1.2, NS; MD 5.3±2.6, NS; HD 31.3±8.8, $P<0.01$) and induced dose-related reductions in the duration of non-social activity (Control 217.4±8.6; LD 231.7±9.7, NS; MD 214.0±10.4, NS; HD 178.4±10.7, $P<0.05$) and social investigation (Control 46.3±3.1; LD 51.4±5.2, NS; MD 54.4±4.6, NS; HD 19.2±8.7, $P<0.01$). Apomorphine increased time spent by the mice in sniffing the sawdust substrate (Control 6.8±1.3; LD 11.4±1.7, $P<0.05$; MD 14.0±3.1, NS; HD 16.3±4.7, NS). However, defensive behaviour was not increased but slightly reduced by apomorphine in females (Control 23.4± 4.8; LD 5.1±1.8, NS; MD 14.2±5.6, NS; HD 6.6±2.3; NS).

It is concluded that although the effects of apomorphine on immobility and non-social and social activities in female mice resemble its effects in males, its effects on defensive behaviour appear to differ between the sexes.

Results expressed as mean duration (s.)±S.E.M. Statistics; Mann-Whitney U and Kruskal Wallis tests. NS = not significant.

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91P THE DOPAMINE UPTAKE INHIBITOR GBR 12909 INDUCES CROSS-TOLERANCE TO RATE-SUPPRESSANT ACTIONS OF COCAINE IN RATS

M. Thabit & A.J. Goudie, Psychology Department, Liverpool University, P.O. Box 147, Liverpool, L69 3BX, U.K.

Cocaine's behavioural effects are believed to be due to the drug's action in inhibiting uptake at the dopamine (DA) transporter (Ritz et al. 1987). Katz et al. (1993) recently reported that, although chronic administration of cocaine to rats induced tolerance to cocaine's suppressant effect on operant responding, it did not induce cross-tolerance to the rate-suppressant action of the specific DA uptake inhibitor GBR 12909 (Andersen, 1989), suggesting that there may be subtle differences between different DA uptake inhibitors (Katz et al. 1993). We have previously reported that chronic GBR 12909 treatment induces cross-tolerance to the cocaine stimulus (Thabit & Goudie, 1992). We therefore extended our studies to see whether GBR 12909 treatment induces cross-tolerance to cocaine's rate-suppressant action. Female Wistar rats (ca. 300 g) were trained to work for food in 20 min daily sessions on a Fixed Ratio 5 schedule. The time- and dose-effect curves (DEC) for GBR 12909-induced suppression of responding were determined initially. GBR 12909 (p.o.) suppressed responding in a dose and time-dependent fashion. The ED_{50} for response suppression was 14.3 mg/kg, and a dose of 15 mg/kg of GBR 12909 (p.o.) suppressed responding significantly when administered at time intervals up to, but not greater than, 120 mins before behavioural testing. Subsequently, the cocaine (i.p.) DEC was assessed before and after 29 days of chronic GBR 12909 (15 mg/kg, p.o., 1 h before operant sessions). GBR 12909 at 15 mg/kg initially suppressed responding by 64%, although complete tolerance developed to this effect over days of treatment. GBR 12909 treatment shifted the ED_{50} for cocaine significantly (repeated measures ANOVA, $p < 0.0005$) from 11.8 to 15.7 mg/kg. In a subsequent study with a different group of rats and a larger dose of GBR 12909, the cocaine DEC was again assessed before and after 20 days of chronic GBR 12909 (20 mg/kg, p.o., 2 h before operant sessions). GBR 12909 at 20 mg/kg initially suppressed responding by 62%. Complete tolerance also developed to this effect during treatment. GBR 12909 treatment again shifted the ED_{50} for cocaine significantly (repeated measures ANOVA, $p < 0.002$) from 13.5 to 23.8 mg/kg. Thus a greater shift in the cocaine DEC was seen after treatment with 20 (1.8 fold shift) rather than 15 (1.3 fold shift) mg/kg of GBR 12909. These data show clearly that GBR 12909 induces cross-tolerance to the rate-suppressant actions of cocaine. They contrast with the findings of Katz et al. (1993), for reasons that are not at present clear. The ability of GBR 12909 to induce cross-tolerance to the behavioural effects of cocaine emphasises the important role that DA uptake inhibition may play in cocaine's actions and in tolerance to cocaine (cf. Thabit & Goudie, 1992).

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92P ANGIOTENSIN II STIMULATES DOPAMINE RELEASE FROM RAT STRIATAL SLICES VIA THE AT_1 RECEPTOR

C. D. Brown, N. M. Barnes, Department of Pharmacology, The Medical School, University of Birmingham, Birmingham, B15 2TT UK.

Angiotensin II (AT) has previously been shown to enhance the release of noradrenaline from rat frontal cortex slices via the AT_1 receptor (Steward et al., 1993). Evidence is also available to suggest that AT may modulate the release of another catecholamine, dopamine (DA), in the striatum (see Barnes et al., 1993 for references). In the present study, we directly assess the ability of AT to modulate the spontaneous release of DA from slices of rat striatum.

Female Wistar rats (190-310 g, Birmingham bred) were killed by cervical dislocation and the striatum removed. Slices of striatum were cut with a McIlwain tissue chopper (400 x 400 µm x thickness of the striatum). Cut tissue was loaded into perfusion chambers and perfused with gassed (95/5 O_2/CO_2) Krebs buffer (mM; NaCl 120, KCl 4.75, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11) plus tyrosine (50 µM), GBR12909 (1.0 µM) and (-)-sulpiride (1.0 µM) for 120 min before the continuous collection of perfusate fractions. DA in the perfusate was quantified by high performance liquid chromatography with electrochemical detection. Protein content was estimated by the Commassie Blue method using bovine serum albumin as standard.

AT (1.0 µM) enhanced the release of DA from the striatal slices (Fig. 1) and this response was antagonised by losartan (1.0 µM; Fig.1).

The present studies indicate that AT modulates the release of DA from rat striatal slices via the AT_1 receptor subtype since the selective AT_1 receptor antagonist, losartan (Timmermans et al., 1991), completely blocked the AT-induced increase in DA release. In addition, preliminary data indicates that the AT_2 receptor ligand, PD123177 (1.0 µM), fails to either modulate endogenous DA release or antagonise the AT-induced stimulation of DA release from striatal slices.

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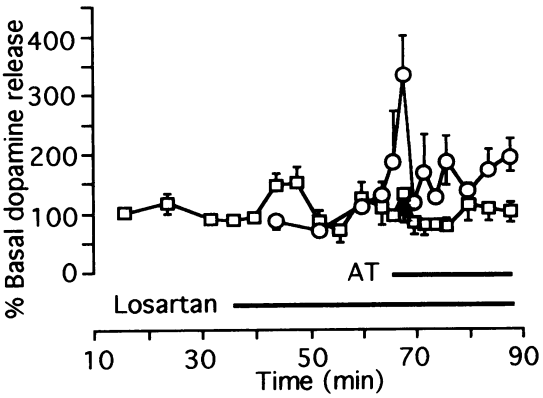

Figure 1. Ability of AT (1.0 µM, ○) and losartan (1.0 µM; AT + losartan, □) to modulate spontaneous DA release from rat striatal slices. Data represent mean±SEM, n = 3-4. Basal DA release = 0.43±0.16 pmol min^{-1} mg^{-1} protein, mean±SEM, n =7.

93P ROPINIROLE, A NOVEL DOPAMINERGIC AGENT FOR THE TREATMENT OF PARKINSON'S DISEASE, WITH SELECTIVITY FOR CLONED DOPAMINE D_3 RECEPTORS

W. P.Bowen, M. C. Coldwell, F. R. Hicks, G. J. Riley & R. Fears, SmithKline Beecham Pharmaceuticals, Harlow, Essex, CM19 5AD.

Ropinirole (SKF 101468) is a non-ergoline dopamine agonist in development for the treatment of Parkinson's disease. Preclinical studies showed that ropinirole was selective for dopamine D2 over D1 receptors (Eden et al., 1991). Identification of the D3 receptor (Sokoloff et al., 1990), led to a sub-division of the D2 classification. Initial reports suggested that some dopamine agonists were D3 selective while others were equipotent at D2 and D3. As this may have important functional consequences, we wished to compare the selectivity of ropinirole with a series of dopamine agonists. The inhibition of [^{125}I]-iodosulpride binding by dopamine agonists to cloned rat (r) and human (h) D2 and D3 dopamine receptors expressed in Chinese Hamster Ovary cells, was as described by Coldwell *et al* (1992). Membranes (5-15 µg protein) were incubated with [^{125}I]-iodosulpride (0.1 nM) in a buffer containing 50 mM Tris (pH 7.4) for 30 min at 37°C. Ascorbic acid (1 µM) was included in incubations with dopamine. Data were analysed using a non-linear least squares fitting procedure.

Table 1. Estimates of Ki (high affinity site; nM) for human and rat D2 and D3 clones.

	rD2	rD3	hD2	hD3
Lisuride	0.39	0.30	0.16	0.19
Bromocriptine	2.6	2.4	3.1	1.9
Quinagolide	3.0	1.2	0.98	0.58
Pergolide	22	1.5	8.0	1.6
Quinelorane	49	0.75	26	1.1
Quinpirole	1100	30	1000	99
Apomorphine	430	190	46	63
Dopamine	670	12	750	44
Ropinirole	950	99	1400	70

Previous radioligand binding studies identified at least two binding sites in each D2 and D3 receptor (Coldwell *et al.*, 1992; Bowen *et al.*, 1993). Using the higher affinity site for comparison, ropinirole was 20-fold selective for the hD3 receptor over the hD2 (Table 1). This was the same order as that found for dopamine, quinpirole and quinelorane. In contrast, apomorphine, lisuride and bromocriptine showed little preference for either receptor.

5'-Guanylylimidodiphosphate (100 µM; GppNHp) produced significant ($p<0.05$, paired t-test) rightward shifts in ropinirole inhibition curves for human D2 and D3 receptors. IC50 values for ropinirole alone were hD2: 1000 ± 91 and hD3: 68 ± 5.5 (mean ± s.e.mean). The shifts with GppNHp were 7.3 and 2.2 fold respectively, resembling those obtained for quinpirole and dopamine under identical conditions, supporting further the similarity of ropinirole and this group of dopamine agonists (Bowen *et al.*, 1993).

We have confirmed that dopamine agonists can have low or high selectivity for the rat and human D3 receptors. The profile of ropinirole resembles that of dopamine, and this may have functional consequences in comparison with compounds from the non-selective group.

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94P D_1 AGONISTS SUPPRESS EPILEPTIFORM ACTIVITY IN THE RAT CORTICAL SLICE

Abdul M. Alam and Michael S. Starr, Department of Pharmacology, School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom.

In a number of in vivo experimental models of epilepsy, the traditional anticonvulsant action of dopamine, mediated by D2 receptor, is balanced by a proconvulsant action of the catecholamine at D1 receptors. This study sought to characterise the effects of D1 agonists on epileptiform activity in vitro.

Coronal wedges of rat brain (500 µm) were placed in a grease-gap chamber so as to confine the corpus callosum and cingulate cortex to separate compartments (Horne et al., 1986). Spontaneous paroxysmal discharges, induced by zero magnesium Krebs, were recorded with Ag/AgCl electrodes. These took the form of an initial population spike (frequency 1-8 per min), followed by a prolonged afterdepolarisation (1-15 s) in 63/103 slices, on which were superimposed further secondary depolarising afterpotentials (SDAPs).

Dopamine (10-1000 µM), applied to the cortex, dose-dependently facilitated SDAP occurrence (+48% at 100 µM) in 2/23 slices, but inhibited SDAPs (ED50 170 µM) and spikes (ED50 1 mM) in 13/23 slices, and exerted mixed excitatory/inhibitory effects in 8/23 slices. Only the inhibitory response was reproduced by selective D1 agonists (5-500 µM), including SKF 38393, SKF 75670, SKF 80723 and SKF 82526. As with dopamine, SDAPs (ED50s 50-70 µM) were suppressed more readily than the primary spikes (ED50s 160-420 µM). The antiepileptic effect of D1 stimulation was mimicked by the adenylyl cyclase activator forskolin (10-100 µM), potentiated by the phosphodiesterase inhibitor IBMX (500 µM), and abolished by the selective D1 receptor antagonists SCH 23390 (0.5 µM) and SCH 39166 (0.5 µM), but not by the beta-adrenoceptor blocking drug propranolol (2 µM). Higher doses of the D1 antagonists (5 µM) were potently antiepileptic by themselves.

These data indicate that dopamine and selective D1 dopamine receptor agonists, suppress the paroxysmal epileptiform discharges induced by zero magnesium in rat cingulate cortex slices. The findings support the antiepileptic activity reported for dopamine and SKF 38393 in isolated hippocampus (Smialowski, 1990; Suppes et al., 1985), and suggest that this is mediated via D1 receptors that are positively coupled to the enzyme adenylyl cyclase.

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95P 5-HYDROXYTRYPTAMINERGIC DRUGS COMPARED ON THE BEHAVIOURAL SEQUENCE ASSOCIATED WITH SATIETY

J.C.G.Halford & J.E.Blundell, Biopsychology Group, Department of Psychology, University of Leeds, Leeds, LS2 9JT, UK.

Cessation of feeding is generally followed by a period of active behaviour and grooming. These in turn subside to be replaced by resting. This has been termed the behavoural satiety sequence (Antin *et al.*1975). This pattern can be used to indicate whether drug induced suppression of eating is associated with a normal satiety profile, and is particularly relevant to serotoninergic drugs because of the proposed role of 5-HT in satiety (Blundell 1977). It has been claimed that fenfluramine retards the onset of resting on the first occasion but not thereafter while fluoxetine retards resting on every subsequent occasion after the first (McGuirk *et al.*1992). Initially it was claimed that the retarding of resting constituted a disruption of the satiety sequence (Willner *et al.*1990) but it is now argued that the behavioural satiety sequence is not a valid indicator of satiety (McGuirk *et al.*1992). Small differences between drugs in the occurrence of particular behaviours may be due to an incomplete sampling technique and it can be questioned whether the postponement of one behaviour (resting) constitutes grounds for asserting the whole sequence has been disrupted. Accordingly two 5-hydroxytryptaminergic drugs have been compared using continuous and exhaustive monitoring of behaviour after feeding.

12♂ Lister hooded rats (250-300g) were housed and monitored individually on a 12h reversed light-dark cycle (LO 0900h). Following a short fast (4h) rats were placed in a familiar observation chamber with wet mash, under red light. Behaviour was exhaustively coded into 8 categories and logged into a PC using a data collection program (*KEETH*) into the following mutually exclusive categories:- *eating, drinking, grooming, locomotion, rearing, sniffing, resting and other.* Equianorectic doses of d-fenfluramine [DF](Sigma, Poole, UK) i.p.1mg/kg and fluoxetine [FX](Lilly, Indianapolis) i.p.10mg/kg dissolved into surgical saline were injected 1h before observation. Each animal was its own control. Treatments were separated by a week to allow metabolite flush out.

Drugs altered the profile oscillations of eating(DF $p<0.05$, FX $p<0.05$), grooming(DF $p<0.05$, FX $p<0.01$), rearing(DF $p<0.01$, FX $p<0.01$), sniffing(DF $p<0.01$, FX $p<0.01$) and resting(DF $p<0.01$, FX $p<0.01$). D-fenfluramine altered the following behaviours by duration(d) and frequency(fq); eating decrease(d $p<0.05$, fq $p<0.01$), locomotion decrease(d $p<0.05$) rearing decrease(fq $p<0.05$) and resting increase(d $p<0.05$). Fluoxetine altered the following behaviours; eating decrease(d $p<0.05$, fq $p<0.001$), grooming decrease(fq $p<0.05$), locomotion decrease(d $p<0.005$, fq $p<0.001$), rearing decrease(d $p<0.05$, fq $p<0.01$) sniffing decrease(fq $p<0.001$) and increase resting (d $p<0.05$). Both drugs gave rise to substantial changes in the total duration and frequency of the behaviour expressed. Both drugs advanced the onset of resting whilst preserving the qualitative pattern of behaviour. Eating suppression is associated with a behavioural profile consistent with the continued operation of the process underlying satiety.

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96P A POTENTIAL INVOLVEMENT OF THE 5-HT_4 RECEPTOR IN BEHAVIOURAL RESPONDING TO AN AVERSIVE SITUATION?

B. Costall, M.E. Kelly & R.J. Naylor, Postgraduate Studies in Pharmacology, School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP

The presence of 5-HT_4 receptors in the brain has not been correlated to a functional role (Dumuis *et al.*, 1988). In the present study we investigate a potential involvement of the 5-HT_4 receptor in behavioural responding to an aversive situation.

Male albino BKW mice (Bradford strain) received an intraperitoneal injection of vehicle, 5-hydroxytryptophan (5-HTP, 50 mg kg^{-1}), ritanserin (1.0 mg kg^{-1}), ondansetron (1.0 mg kg^{-1}), SDZ205-557 (2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino) ethyl ester, 0.01 mg 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 centre of the brightly illuminated compartment of a two compartment light/dark test box and the time spent in the two chambers was recorded by video camera over a 5 min period (for methodological details see Costall et al, 1989); % time spent in the light area is shown in Table 1.

Table 1. The action of 5-HTP and its interaction with 5-HT receptor antagonists to inhibit and disinhibit mouse behaviour in the light/dark test box.

Treatment	% Time in Light	Treatment	% Time in Light
Vehicle	42 ± 3.8	5-HTP + Ritanserin	80 ± 6.5*
5-HTP	18 ± 1.4*	5-HTP + Ondansetron	42 ± 5.3
Ritanserin	51 ± 5.3	5-HTP + SDZ205-557	14 ± 1.8*
Ondansetron	43 ± 4.1	5-HTP + Ritanserin + Ondansetron	77 ± 5.4*
SDZ205-557	40 ± 5.2	5-HTP + Ritanserin + SDZ205-557	33 ± 3.8^{+}

n = 10. A significant decrease or increase compared to the vehicle response is shown *P<0.001; a significant antagonism compared to 5-HTP + Ritanserin is shown $^{+}$P<0.001 (one way ANOVA followed by Dunnett's t test).

The 5-HTP induced decrease in behaviour in the light compartment of the test box reflects an anxiogenic profile (Costall *et al.*, 1989), its dominant effect, which is antagonised by the 5-HT_{1C}/5-HT_2 receptor antagonist ritanserin and the 5-HT_3 receptor antagonist ondansetron. The antagonism afforded by ritanserin reversed the action of 5-HTP to an increased exploration of the light compartment above that of the vehicle treated controls. This was inhibited by the 5-HT_3/5-HT_4 receptor antagonist SDZ205-557 (Buchheit *et al.*, 1991; Eglen *et al.*, 1993) but not by the 5-HT_3 receptor antagonist ondansetron. The data are supportive of hypotheses that 5-hydroxytryptamine may normally act to inhibit behavioural responding. However, a disinhibitory potential is also revealed by the use of ritanserin and its antagonism by SDZ205-557 but not ondansetron, which tentatively indicates a 5-HT_4 receptor involvement in mouse responding to drug induced changes in behaviour to the aversive situation of the light/dark box.

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97P INVOLVEMENT OF 5-HT_{1A} RECEPTORS IN MORPHINE ANTINOCICEPTION IN RATS AND MICE: A POSSIBLE SPECIES DIFFERENCE

M.A. Ashworth-Preece, J.E. Hartley and A. Fletcher. Wyeth Research (UK) Ltd., Taplow, Maidenhead, Berkshire, SL6 0PH.

Systemically administered 5-HT_{1A} agonists have been reported to induce analgesia, hyperalgesia, or to have no effect upon pain thresholds (Millan et al., 1991). In addition, it has been reported that 5-HT_{1A} receptors play an important role in the expression of morphine analgesia (Hammond., 1990). In view of reported species differences in 5-HT_{1A} receptor mediated effects (Bill et al., 1991), the present study examined the effect of the 5-HT_{1A} receptor agonist 8-hydroxy-N,N-dipropylamino-tetralin (8-OH-DPAT) and the 5-HT_{1A} receptor partial agonist gepirone on both tonic nociceptive thresholds and morphine-induced antinociception in the rat and mouse hot plate and radiant heat tail flick paradigms. In addition, the 5-HT_{1A} receptor antagonists (±)-WAY-100135 and (+)-WAY-100135 (Fletcher et al., 1993) were tested in the rat and mouse hot plate paradigm. Male Sprague-Dawley rats (220 - 440 g) or female Tuck T/O mice (20 - 30g) were co-injected subcutaneously with vehicle, 8-OH-DPAT (0.1 - 0.3 mg/kg), gepirone (1 - 3 mg/kg), (±)-WAY-100135 (3 - 10 mg/kg) or (+)-WAY-100135 (3 - 10 mg/kg) and vehicle or morphine (3 - 10 mg/kg). Thirty minutes later, latencies to respond in either the hot plate or radiant heat tail flick were assessed (n = 8 per treatment) and expressed as percentage maximum possible effect ((postdrug latency - predrug latency / cut-off time - predrug latency) X 100) (%MPE) (Yaksh et al., 1979). None of the 5-HT_{1A} receptor ligands had any significant effect upon tonic nociceptive thresholds in either species. In the rat hot-plate, 8-OH-DPAT and gepirone significantly ($p < 0.01$) attenuated morphine induced antinociception, reducing %MPE from 84.0 ± 9.5 (10 mg/kg morphine/vehicle) to 5.1 ± 1.5 (10 mg/kg morphine/0.1 mg/kg 8-OH-DPAT) and from 66.4 ± 13.4 (10 mg/kg morphine/vehicle) to 18.8 ± 6.4 (10 mg/kg morphine/3 mg/kg gepirone). In the rat tail flick, 8-OH-DPAT also significantly ($p < 0.01$) attenuated morphine induced antinociception, reducing %MPE from 100 (3 mg/kg morphine/vehicle) to 61.6 ± 9.2 (3 mg/kg morphine/0.1 mg/kg 8-OH-DPAT). In the mouse hot plate and tail flick, neither 8-OH-DPAT or gepirone had any effect upon morphine induced antinociception. Neither (±)-WAY-100135 or (+)-WAY-100135 had any effect upon morphine-induced antinociception in either the rat or mouse hot plate paradigm. These data indicate that 5-HT_{1A} receptor agonists attenuate morphine-induced antinociception in the rat but not in the mouse. Evidence therefore exists for a species difference in respect of the involvement of 5-HT_{1A} receptor mediated mechanisms in morphine-induced antinociception which is consistent with previous evidence of species differences in 8-OH-DPAT-induced hypothermia (Bill et al., 1991). In addition, the lack of effect of the selective antagonist (+)-WAY-100135 on nociception and opiate analgesia suggests that there is no 5-HT_{1A} receptor-mediated tonic influence on pain thresholds.

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98P EFFECTS OF GEPIRONE AND BUSPIRONE ON OPERANT FOOD INTAKE IN NON-DEPRIVED RATS

I.S. Ebenezer. Neuropharmacology Research Group, School of Pharmacy, University of Portsmouth, Portsmouth, PO1 2DZ.

It has previously been shown that drugs, such as 8-OH-DPAT, that display agonist properties at central 5HT_{1A} receptors increase food intake in free feeding rats (see Ebenezer,1992). Recently, it has also been demonstrated that 8-OH-DPAT will increase operant responding for food in non-deprived rats, and this observation supports the view that the hyperphagic effect of the drug is behaviourally specific, and independent of phenomena such as drug induced chewing (Ebenezer, 1992). The present study was carried out to investigate the effects of two other 5HT_{1A} agonist gepirone and buspirone on operant food intake in rats.

Experiment 1. Male Wistar rats (n=6, b.wt. 330 - 420g) were trained to press a lever in an operant conditioning chamber for food pellets on a fixed ratio of 4 (FR-4), as described previously (Ebenezer, 1992). Thereafter the rats were fed ad libitum in their home cages and given 6 further daily training sessions in the operant chamber to press the lever for food on the FR-4 schedule. During experimental sessions the non deprived rats were injected s.c. with either physiological saline or gepirone (0.25 - 2 mg kg^{-1}) and placed separately in the operant chamber for 90 min. and operant food intake was measured during this period. A repeated measures design was used with each rat receiving all doses of gepirone.

Experiment 2. Male Wistar rats (n=6, b.wt. 330 - 410g) were trained as described for Experiment 1, and a similar experimental protocol was used. The rats were injected s.c. with physiological saline or buspirone (0.25 - 1.0 mg kg^{-1}) and operant food intake measured for 90 min.

The results obtained in Experiment 1 and 2 are shown in Tables 1and 2 respectively.

Table 1. Effects of gepirone (G) on operant food intake.

Treatment	Mean Food Intake (g) ± s.e.mean
Saline	3.66 ± 0.6
G (0.25 mg kg^{-1})	5.15 ± 0.5*
G (0.5 mg kg^{-1})	4.97 ± 0.7*
G (1.0 mg kg^{-1})	5.05 ± 0.5*
G (2.0 mg kg^{-1})	4.81 ± 0.4*

Table 2. Effects of buspirone (B) on operant food intake

Treatment	Mean Food Intake (g) ± s.e. mean
Saline	4.10 ± 0.6
B (0.25 mg kg^{-1})	6.11 ± 0.5*
B (0.5 mg kg^{-1})	5.99 ± 0.7*
B (1.0 mg kg^{-1})	2.89 ± 0.5

[*P<0.05]

The results of this study extend previous findings (Ebenezer, 1992), and show that both gepirone and buspirone will increase operant food intake in rats. The reduction in food intake to below control levels after buspirone (1.0 mg kg-1) may be due to the fact that buspirone also has dopaminergic antagonist activity (Wood et al., 1983).

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J.A. Rudd, K.T. Bunce[1] & R.J. Naylor. Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, BD7 1DP. [1]Dept. of Gastrointestinal Pharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 ODP.

The 5-HT$_{1A}$ receptor agonists buspirone, 8-OH-DPAT (8-hydroxy-2(di-n-propylamino)-tetralin) and flesinoxan have been reported to have antiemetic efficacy against motion-induced and drug-induced emesis in the cat (see Lucot, 1992). 8-OH-DPAT has also been reported to differentially antagonise drug-induced emesis in the ferret (Rudd *et al.*, 1992). In the present study we evaluate the potential of buspirone, flesinoxan (Fles), lesopitron (Lesop) and gepirone (Gep) to antagonise copper sulphate-induced emesis in the ferret.

Ferrets (either sex; 0.7-1.4 kg) were administered buspirone, Fles, Lesop, Gep (0.05-1.0 mg kg^{-1}) or vehicle (Veh; saline 0.9 % w/v) subcutaneously as a 15 min pretreatment prior to the administration of $CuSO_4.5H_2O$ (100 mg kg^{-1} intragastric). The latency to the first retch or vomit and the total numbers of retches and vomits were recorded over the subsequent 30 min observation time.

Table 1 The effect of buspirone, flesinoxan, lesopitron and gepirone on the retching and vomiting induced by intragastric copper sulphate

Treatment	mg/kg	Latency (min)	Retches	Vomits	RV/T	Treatment	mg/kg	Latency (min)	Retches	Vomits	RV/T
Veh		4.0	64.5±6.7	8.7±1.4	6/6	Veh		3.2	79.0±6.8	8.0±0.3	7/7
Buspirone	0.25	4.4	48.8±12.8	8.3±1.9	4/4	Lesop	0.05	4.2	53.8±9.5	8.3±1.1	4/4
	0.5	9.0	16.8±5.3*	3.0±1.4*	4/4		0.1	5.4	38.8±14.6*	6.3±1.0*	4/4
	0.75	10.4	20.8±6.1*	4.5±1.7	4/4		0.25	5.3	46.3±9.9*	4.5±1.2*	4/4
	1.0	4.4	53.3±7.3	9.0±0.7	4/4		0.5	8.1	68.8±18.4	7.2±1.2	4/5
Veh		3.8	38.3±7.8	4.3±0.7	6/6	Veh		4.6	75.8±7.8	7.3±1.3	6/6
Fles	0.25	5.6	37.8±4.6	6.3±0.5	4/4	Gep	0.25	7.4	39.5±8.8*	6.3±1.4	4/4
	0.5	7.0	26.0±11.4	5.0±1.6	4/4		0.5	5.5	21.3±7.2*	2.8±1.3*	4/4
	0.75	6.1	12.0±5.3*	1.0±0.4*	3/4		0.75	5.0	30.3±15.2	4.8±1.8	4/4

Results represent the mean±s.e.mean for the numbers of retches and vomits; the mean latency to the first retch or vomit is indicated in min; RV/T indicates the numbers of animals either retching or vomiting out of the numbers of animals tested. Significant differences between Veh and drug treated animals are indicated as *P<0.05 (Mann-Whitney U test).

All 5-HT$_{1A}$ receptor agonists except flesinoxan displayed a U-shaped dose-response profile to antagonise copper sulphate-induced emesis and produced maximum inhibitions of up to 70-80%. The latency to first retch or vomit was also increased by all agonists tested.

It is concluded that an agonist action at the 5-HT$_{1A}$ receptor may suppress emesis induced by gastric irritation in the ferret. The site(s) of antiemetic action is unknown but may be mediated centrally since the emesis to morphine and apomorphine has been suppressed by 8-OH-DPAT (Rudd *et al.*, 1992).

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100P ALOSETRON ATTENUATES REBOUND HYPERACTIVITY INDUCED BY WITHDRAWAL FROM A CHRONIC MESOLIMBIC DOPAMINE INFUSION AND SYSTEMIC NEUROLEPTIC TREATMENT

A.G. Smith, A.M. Domeney, B. Costall, R.J. Naylor & M.B. Tyers[1], Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP, and [1]Pharmacology Division, Glaxo Group Research Ltd., Ware, Hertfordshire, SG12 ODJ

The selective 5-HT$_3$ receptor antagonist alosetron has previously been shown to potently reduce the hyperactivity response resulting from a period of raised mesolimbic dopaminergic activity in the rodent, suggesting a potential antipsychotic profile of this compound (Domeney et al., 1992). In a clinical situation however it is likely that patients requiring antipsychotic therapy may have previously received or still be receiving treatment with neuroleptic agents such as haloperidol. Such drug history may thus compromise the efficacy of subsequent treatment regimens. The aim of the present studies was to investigate the ability of alosetron to both prevent the development of withdrawal hyperactivity and to suppress an ongoing enhanced psychomotor drive induced by withdrawal from a 12 day intra accumbens dopamine infusion with concomitant neuroleptic treatment.

Sprague-Dawley rats (Bantin & Kingman, Hull; 12 weeks old and weighing 300 ± 25g) were subject to standard stereotaxic surgery for the implantation of chronically indwelling guide cannulae for the subsequent infusion of dopamine (50µg/24h) or vehicle (0.1%, $Na_2S_2O_5$, 0.48µl/h) into the centre of the nucleus accumbens for 12 days. Concurrent with the dopamine infusion rats received haloperidol 0.3mg/kg twice daily by the intraperitoneal route (8 am and 5 pm). This treatment suppressed both the hyperactivity response to dopamine and reduced locomotor activity to below the level of control, vehicle-treated animals. When combined dopamine and haloperidol treatment were withdrawn on day 12 rebound hyperactivity developed within 2-3 days and persisted for up to 14 days (250 ± 14.3 mean total counts) compared with vehicle control values of 125 ± 14.2 mean total counts ($P<0.001$). Data is expressed as mean daily activity counts calculated over an 18 day withdrawal period. Alosetron (100µg/kg i.p. given twice daily throughout the period of dopamine infusion/neuroleptic treatment) prevented the development of withdrawal hyperactivity (145 ± 16.4 mean total counts) compared to dopamine plus haloperidol control values of 250 ± 14.3 mean total counts ($P<0.001$). In addition, alosetron (100µg/kg i.p.) given twice daily to animals in which a withdrawal hyperactivity response had developed significantly ($P<0.001$), attenuated the hyperactivity (160 ± 12.3 mean total counts compared to 250 ± 14.3 mean total counts, dopamine plus haloperidol control). Further, animals withdrawn from a dopamine infusion in which alosetron was given concomitantly throughout the dopamine infusion showed levels of locomotor activity that were not significantly different ($P>0.05$) from those of control, vehicle-treated animals during the withdrawal period.

It is concluded that alosetron not only has the ability to inhibit locomotor hyperactivity resulting from a raised mesolimbic dopamine function in the rodent, but is also able to control the hyperactivity following withdrawal from a period in which mesolimbic dopamine excess has been suppressed with haloperidol.

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101P INTERACTION BETWEEN 5-HYDROXYTRYPTOPHAN AND 5-HYDROXYTRYPTAMINE RECEPTOR ANTAGONISTS TO INHIBIT AND DISINHIBIT RAT SOCIAL INTERACTION

B. Costall, M.E. Kelly & R.J. Naylor, Postgraduate Studies in Pharmacology, School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP

The ability of 5-hydroxytryptophan (5-HTP) to exacerbate the behavioural response of mice to the aversive situation of the light/dark test box is reversed by ritanserin to reveal a disinhibitory potential (Costall et al., 1993). The present study was designed to investigate whether the inhibitory and disinhibitory effects of 5-HTP could also be observed in the rat.

Male Lister Hooded rats (250-300g) were injected intraperitoneally with vehicle, 5-HTP (50 mg kg^{-1}), ritanserin (1.0 mg kg^{-1}), ondansetron (1.0 mg kg^{-1}), and SDZ205-557 (2-methoxy-4-amino-5-chloro-benzoic acid 2-(diethyl amino) ethyl ester) as 40 min pretreatments according to the regimens shown on Table 1, with testing 40 min after the last treatment. Changes in rat social interaction and exploratory behaviour were measured by placing naïve pairs of rats into an open topped box with floor markings and recording behaviour over a 10 min period by remote video camera. Social interaction was determined by timing the sniffing of partner, crawling under or climbing over partner, genital investigation of partner and following partner, and locomotor exploration as the number of line crossings.

Table 1. The effect of 5-HTP and its interaction with 5-hydroxytryptamine (5-HT) receptor antagonists to modify rat social interaction and locomotor activity under conditions of unfamiliarity and high illumination.

Treatment	Social Interaction(s)	Line Crossings	Treatment	Social Interaction(s)	Line Crossings
Vehicle	58 ± 6	114 ± 14	5-HTP + Ritanserin	123 ± 11*	119 ± 14
5-HTP	25 ± 2*	112 ± 10	5-HTP + Ondasetron	65 ± 6	123 ± 16
Ritanserin	54 ± 7	110 ± 9	5-HTP + SDZ205-557	60 ± 5	112 ± 14
Ondansetron	54 ± 8	117 ± 14	5-HTP + Ritanserin + Ondansetron	109 ± 13*	105 ± 11
SDZ205-557	51 ± 7	108 ± 7	5-HTP + Ritanserin + SDZ205-557	40 ± 4.9+	110 ± 9

n = 6 pairs; values are the mean ± s.e. mean. A significant decrease or increase compared to the vehicle control is shown *P<0.001; a significant antagonism compared to 5-HTP + Ritanserin is shown +P<0.001 (one way ANOVA followed by Dunnett's t test).

The dominant effect of 5-HTP is to decrease social interaction, an anxiogenic profile, which is antagonised by the 5-HT_{1C}/5-HT_2 receptor antagonist ritanserin and the 5-HT_3 receptor antagonist ondansetron. Ritanserin reversed the action of 5-HTP to increase social interaction above that of vehicle treated controls; this was inhibited by the 5-HT_3/5-HT_4 receptor antagonist SDZ205-557 (Buchheit et al., 1991; Eglen et al., 1993) but not by ondansetron. Locomotor activity was not modified by any treatment. In agreement with results reported in the mouse (Costall *et al.*, 1993), the present data indicates that 5-HT may normally act to inhibit behavioural responding but that a disinhibitory potential, as revealed by ritanserin and antagonised by SDZ205-557, indicates a 5-HT_4 receptor involvement in releasing behaviour suppressed by the aversive conditions of the rat social interaction test.

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102P (–)-FLUOXETINE HAS HIGH AFFINITY FOR THE CLONED RAT AND HUMAN 5-HT_{1C} RECEPTOR AND THE HUMAN 5-HT_2 RECEPTOR

M D Wood, A Glen, T P Blackburn, J A Lee*, J A Sutiphong*, C Kumar*, J Carey +, J Robinson +SmithKline Beecham Pharmaceuticals, Coldharbour Road, Harlow, Essex CM19 5AD*, King of Prussia, Pennsylvania, USA,
+ Yew Tree Bottom Road, Epsom, KT18 5XQ

Fluoxetine is a member of the selective serotonin reuptake inhibitors (SSRIs). However, fluoxetine inhibits the [^{3}H]-mesulergine (Mes) binding in the bovine choroid plexus to a 5-HT_{1C} site (Wong et al, 1991). Although this inhibition was seen at near micromolar concentrations (pK_I 6.57) this was performed in a species different to that used in determining inhibition of 5-HT uptake (rat, pK_I 7.53). We have therefore studied the interaction of fluoxetine and its enantiomers with the rat and human cloned 5-HT_{1C} receptor and the closely related human 5-HT_2 receptor.

Binding of [^{3}H]-Mes and [^{3}H]-ketanserin (both 0.5 nM) was performed in membranes prepared by homogenising whole cell pellets. Specific binding was that displaced by 10^{-6} M mianserin. Saturation data was analysed using LIGAND inhibition studies ALLFIT (4-parameter logistic). The human 5-HT_{1C} receptor (Carey et al, 1992) and the human 5-HT_2 receptors (Saltzman et al, 1991) were expressed in stable HEK 293 cell lines and the rat 5-HT_{1C} receptor expressed transiently also in HEK 293 cells.

In 5-HT_{1C} cell lines, [^{3}H]-Mes bound with high affinity [K_D 0.48 nM ± 0.12 (4) {mean ± SEM} and 0.5 nM ± 0.18 (3) for rat and human clones respectively] and to a single class of sites. Fluoxetine inhibited binding with a pK_I of 7.45 ± 0.09 (4) compared to 6.09 ± 0.06 (3) and 7.97 ± 0.04 (3) for the (+) - and (-) - enantiomers respectively. In the human 5-HT_{1C} receptor clone, fluoxetine inhibited [^{3}H] - Mes binding with a pK_I of 6.91 ± 0.06 (3) compared to 7.61 ± 0.05 for the (-) isomer. Fluoxetine was also a potent inhibitor of [^{3}H] - ketanserin binding to the cloned human 5-HT_2 receptor (Saltzman et al, 1991) with a pK_I of 6.88 ± 0.04 (3) compared to 7.03 ± 0.04 (3) for (-) - fluoxetine.

These results show that fluoxetine is not a selective serotonin reuptake inhibitor in that it inhibits [^{3}H] - Mes binding to the cloned rat and human 5-HT_{1C} and [^{3}H] - ketanserin binding to the cloned human 5-HT_2 receptor at concentrations similar to those needed to block 5-HT uptake. Further to the 5-HT_{1C} activity resides mainly in the (-) isomer.

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103P COMBINED PARA-CHLOROPHENYLALANINE AND MUSCARINIC ANTAGONIST TREATMENT PRODUCES A DEFICIT IN WATER MAZE ACQUISITION

J.A. Harder, M.E. Kelly, C.H.K. Cheng & B. Costall, Studies in Pharmacology, University of Bradford, Bradford, West Yorkshire, BD7 1DP

It has been suggested that the loss of serotonergic (5-HT) neurones found in Alzheimer's disease may act concurrently with a cholinergic dysfunction to produce cognitive impairment (Richter-Levin & Segal, 1989). In the present studies a combined para-chlorophenylalanine (pCPA) and muscarinic antagonist treatment has been investigated for effects on spatial learning in the rodent using the water maze. To confirm that pCPA effects were due to selective 5-HT depletion, low doses of pCPA were administered and HPLC-ECD analysis of brain 5-HT and catecholamine levels was also performed.

Male Lister Hooded rats were given 100mg/kg pCPA i.p. or saline daily for 3 days. On the final treatment day, training in the water maze was initiated for 4 trials per day over 4 days (Harder *et al.*, 1992). Scopolamine (0.25mg/kg i.p.) or atropine (10mg/kg i.p.) were given as 20 or 30 min pretreatments prior to the first trial on each training day. Groups of pCPA and vehicle (saline) treated animals were killed for HPLC determination of hippocampal and cortical indoleamine and catecholamine levels on the final day of pCPA treatment (equivalent to test day 1), on test day 4 and on the day after the completion of testing (day 5).

Table 1: Combined effect of pCPA and muscarinic antagonists on water maze acquisition

Treatment	day one	day two	day three	day four
vehicle	50.1 ± 4.6	44.6 ± 8.9	23.9 ± 4.7	21.6 ± 5.5
pCPA	71.3 ± 11.3	30.0 ± 5.4	18.9 ± 3.3	24.6 ± 7.6
scopolamine	67.6 ± 9.0	40.7 ± 8.2	31.4 ± 5.0	14.0 ± 2.7
pCPA + scopolamine	95.1 ± 2.7+***	82.3 ± 6.8+++***	58.5 ± 12.1+***	33.8 ± 10.9
vehicle	53.6 ± 9.1	37.0 ± 7.5	25.5 ± 4.6	14.1 ± 2.9
pCPA	73.5 ± 8.6	34.7 ± 6.5	19.8 ± 3.6	6.8 ± 0.9
atropine	86.7 ± 7.9**	53.8 ± 7.0	51.3 ± 11.0*	48.4 ± 12.5*
pCPA + atropine	95.3 ± 4.7***	80.1 ± 9.0***+	80.5 ± 7.7***+	54.2 ± 10.8***

Each data represents the mean latency to find island (s) ± s.e. mean, n = 7-8. $^{*+}P<0.05$, $^{**++}P<0.01$, $^{***+++}P<0.005$ Significant differences from *vehicle and from $^{+}$scopolamine/atropine alone (ANOVA followed by Dunnett's t test).

On the day of the final dose of pCPA, 5-HT levels in the pCPA treated rats were significantly reduced by 63% and 71% in the hippocampus and cortex respectively compared to vehicle treated control rats (n = 6, $P<0.01$, 2 tailed Student's t-test). 5-HT remained significantly depleted in the hippocampus (83%) and cortex (88%-89%) on test day 4 and day 5 (n = 6, $P<0.01$).

Doses of scopolamine and atropine which had a limited effect on acquisition caused marked impairment in 5-HT depleted animals. HPLC data suggest that with a 3 day, low dose pCPA treatment regimen, 5-HT is depleted for the duration of a 4 day testing period, while noradrenaline and dopamine levels remain unaffected. These behavioural results thus confirm the data of Richter-Levin & Segal (1989) and demonstrate that the effects observed can be achieved when 5-HT is depleted selectively. A decrease in 5-HT transmission, while having no effect alone, could be important for cognitive function when a concurrent cholinergic deficit exists.

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104P EFFECT OF THE STABLE TRH ANALOGUE RX77368 (pGlu-His-3,3'-dimethylProNH_2) ON TWO COGNITIVE TESTS IN THE SENESCENT RAT

C.D. Watson, K.C.F. Fone, H.C. Jackson[1] & G.W. Bennett. Department of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and [1]CNS Division, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Malov, Denmark.

The tripeptide, thyrotrophin-releasing hormone (TRH) and its more metabolically stable TRH analogues enhance central cholinergic function and attenuate a scopolamine-induced passive avoidance deficit in rodents (Yamamura *et al.*, 1991), scopolamine-induced amnesia and the deficit in cognitive performance seen in Alzheimer's disease (Molchan *et al.*, 1992). Senescent rodents exhibit a decline in cholinergic function similar to that seen in human dementia disorders and so have been used to model the cognitive deficits seen in such disorders (Hagan & Morris, 1988). The present study evaluated whether pretreatment with the stable TRH analogue RX77368 could reverse a senescent learning deficit in rats, assessed using two reinforcement learning paradigms; a one trial passive avoidance (PA) test and a water maze (WM) test.

Male Hooded Lister rats were assigned to 4 groups (A to D: n=9 each) as follows; group A: adult (4 months old) and groups B to D: senescent (23 to 24 months), which performed all tests. All groups were pretreated (-30min i.p.) with either saline (groups A and B; 1mlkg^{-1}) or RX77368 (C and D: 1 and 3mgkg^{-1} respectively). The PA test measured latency (for ≤ 600s) to cross over from the light to dark compartment in a skinner box, in one training (day 1, drug treatment and 0.5mA footshock for 0.5s) and one retention (day 2, no drug treatment or footshock) trial. Photocell activity cages were used to assess locomotor activity (for 600s on day 3 and 4) using a similar protocol to the PA test. On days 5 to 16 the latency to reach a hidden platform in a WM (2 x 180s trials per alternate day followed by 30s on the platform) were recorded. Immediately after the last WM trial (day 16), the time to reach a visible platform (Visual Acuity), time spent within a 50cm radius of the platform with the platform removed (Transfer Test) and swim speeds were assessed.

In PA testing, all treatment groups showed a significant increase ($p<0.001$, Kruskall Wallis followed by multiple comparisons test) in cross over latency in the retention compared with the training trial but senescent controls had a significantly ($p<0.05$) shorter cross over latency in the retention trial than adult controls. In retention trials, senescent rats treated with 1 (582s median latency) but not 3mgkg^{-1} (296s) RX77368 showed an increased cross over latency comparable to adult controls (600s), which itself was significantly ($p<0.05$) longer than that of the senescent controls (331s). The effects of RX77368 on PA are unlikely to result from a change in locomotor activity since photocell activity counts were comparable in all three senescent groups on both days tested. Irrespective of age or treatment, all groups showed a progressive and significant ($p<0.001$, Freidman's ANOVA) decrease in the time to reach the hidden platform in the WM (from >175s on trial 1 to <34s on trial 12). However, compared with adult controls the performance of all three senescent groups was significantly impaired ($p<0.001$) even following either RX77368 pretreatment. In the transfer test senescent rats treated with RX77368 (3mgkg^{-1}), spent significantly ($p<0.01$) less time (14s) within 50 cm of the removed platform than adult (29s), aged (19s) controls or 1mgkg^{-1} RX77368 (19s) groups. None of these deficits were associated with a visual or motor impairment since visual acuity (<18s) and swim speeds (between 62 and 79 squaresmin^{-1}) were similar for all groups.

The use of two reinforcement cognitive tests confirm that there is a decline in performance with senescence in the rat. The TRH analogue RX77368 only improved performance of senescent rats in the negatively reinforced non-spatial task and not in the positively reinforced spatial task.

CDW is a SERC CASE student with Reckitt and Colman who kindly supplied RX77368.
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105P COGNITIVE DEFICITS FOLLOWING CAROTID OCCLUSION IN THE RAT IN THE ABSENCE OF CA_1 CELL DEATH

S. Hogg, B. Costall, M.E. Kelly, J.R. McCurrie & A. Salako, Studies in Pharmacology, School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP

The deficit in cognitive function following cerebral ischaemia has been attributed to the degeneration of hippocampal CA_1 neurones (Auer *et al.*, 1989). In a previous report we have shown that a loss in spatial learning ability is observed as a result of a 45 min period of bilateral common carotid artery occlusion which was not accompanied by a loss in hippocampal CA_1 pyramidal cells (Hogg *et al.*, 1991). However, in that study the surgery was performed under ketamine anaesthesia which is known to be neuroprotective (Marcoux *et al.*, 1988). Here we report the results of a study to establish the effects of a 45 min period of bilateral common carotid artery occlusion performed under either ketamine or isoflurane anaesthesia on learning ability and histological integrity.

Bilateral common carotid artery occlusion (45 min) was performed in adult male Lister-hooded rats under either ketamine (100 mg kg^{-1} i.p.) or isoflurane (in O_2/N_2O carrier) anaesthesia. Seven days after surgery rats were either tested for 4 days (6 trials/day) in the Morris water maze or sacrificed for histological analysis. Brain sections (7 μm) at the level of the anterior hippocampus (Bregma - 3.8 mm) were stained with haematoxylin and eosin. Cell number/unit area was assessed for the CA_1 area using light microscopy techniques and was expressed as a % of the sham-operated control cell count. Learning ability was assessed as the latency (s) to perform the water maze task.

It was observed that the extent of the deficit in spatial learning ability (increased latency) was the same for occluded rats from both the ketamine and the isoflurane treated groups ($P>0.05$). Mean latency scores for sham and occluded rats were 17.3 ± 2.4 and 54.6 ± 3.4 s (ketamine, n = 8, $P<0.001$) and 19.4 ± 2.1 and 49.8 ± 2.9 s (isoflurane n = 8, $P<0.001$). Analysis of the density of CA_1 neurones demonstrated 109.9±0.7% (ketamine, n = 4, $P>0.05$) and 93.3±1.2% (isoflurane, n = 4, $P>0.05$) intact cells when occluded tissue was compared to that obtained from the sham operated controls.

Data from this study demonstrate that a 45 min period of bilateral common carotid artery occlusion in the rat results in a comparable deficit in spatial learning following surgery performed under either ketamine or isoflurane induced anaesthesia. Neither group of sham and occluded rats showed significant changes in the density of hippocampal CA_1 neurones. The mechanism underlying the disturbances in learning ability following bilateral common carotid artery occlusion does not appear to involve a loss in CA_1 neurones. However, since the learning deficit is transient (up to 28 days, Hogg *et al.*, 1992) this finding may not be contradictory to the report by Auer *et al.* (1989).

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106P NEUROTROPHIC FACTORS ENHANCE REGENERATION AND ELEVATE GROWTH-ASSOCIATED PROTEIN mRNAs IN ADULT SENSORY NEURONES

L. Mohiuddin, K. Fernandes, D.R. Tomlinson & P. Fernyhough, Department of Pharmacology, Queen Mary & Westfield College, Mile End Road, London E1 4NS, U.K.

Brain derived neurotrophic factor (BDNF), which has extensive homologies with nerve growth factor (NGF), has trophic effects on sensory neurones as well as on neurones of central nervous system (Thoenen, 1991). These two factors enhance regeneration in cultured adult rat sensory neurones. The aim of this study was to determine the effect of NGF, BDNF and neurotrophin-3 (NT-3; another member of NGF, BDNF gene family) on axonal regeneration and relate this measurement to the levels of gene expression of the axoplasmic growth associated protein, GAP-43 and tubulin Tα1 in dissociated cultures of sensory neurones from dorsal root ganglia of adult, male Wistar rats (200 gm body weight). Cultures were maintained in defined F12 medium in the absence of any form of serum. Morphometric analysis of neurite outgrowth was used to assess the level of regeneration, after which total RNA was isolated and subjected to Northern blotting. The filters were probed sequentially using [^{32}P]-labelled cDNA probes for GAP-43 and tubulin Tα1. A truncated synthetic "sense" cRNA for GAP-43 (NaeI fragment) was included at the start of RNA extraction to enable accurate quantification of mRNA levels. Hybrids were detected by autoradiography and quantified using an image analyser. Hybridisation signals were normalised to the internal standard and adjusted for cell numbers. Time course experiments for effects of NGF on regeneration, GAP-43 and tubulin Tα1 mRNA levels were performed. In response to NGF at 10ng/ml, GAP-43 mRNA levels were increased (over controls) to 1.27 ± 0.29 fold on day 1, 1.44 ± 0.13 fold on day 2 and 1.51 ± 0.13 fold on day 3 (values are mean ± SEM; n = 3-7 separeate experiments; all changes were at least $p<0.05$). Tubulin Tα1 mRNA levels were increased (over controls) to 1.65 ± 0.05 fold on day 1, 2.26 ± 0.55 fold on day 2 and 1.93 ± 0.47 fold on day 3 (changes were at least $p<0.05$). These results were associated with enhanced regeneration. NGF and NT-3 enhanced regeneration and growth associated protein mRNAs in a dose-dependent manner. For example, NGF dependent elevation of regeneration at 2 days with reference to control (i.e. without NGF growth = 1) was 1.7 ± 0.15 (NGF = 0.1 ng/ml), 4.4 ± 0.76 (NGF =1.0 ng/ml) and 7.2 ± 0.32 (NGF = 10.0 ng/ml), compared with up regulation of GAP-43 mRNA levels at 2 days, control = 1.0 (no NGF), 1.54 ± 0.27 (NGF = 0.1 ng/ml), 1.67 ± 0.27 (NGF = 1.0 ng/ml), 2.08 ± 0.33 (NGF = 10.0 ng/ml) (all values are means ± SEM, n=4 separate experiments). The estimated ED_{50} values for regeneration and growth associated protein mRNA up regulation for NGF and NT-3 were above 1.0 ng/ml. However, BDNF elevated regeneration maximally at 1.0 ng/ml with an estimated ED_{50} of 0.3ng/ml. Where full data is available, these results show that neurotrophic factor dependent enhancement of regeneration correlated with up regulation of GAP-43 and tubulin mRNAs (r^2 values were, for regeneration *versus* GAP-43 with NGF = 0.84 and with NT-3 = 0.92 and for regeneration *versus* tubulin = 0.78; $p<0.05$ in all cases).

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107P ANXIOSELECTIVE EFFECTS OF BRETAZENIL (RO16-6028) IN THE MURINE ELEVATED PLUS-MAZE: COMPARISON WITH CHLORDIAZEPOXIDE

R.J. Rodgers* and J.C. Cole, Ethopharmacology Laboratory, Department of Psychology, University of Leeds, Leeds LS2 9JT, U.K.

The side-effect profile of benzodiazepine anxiolytics has led to the search for safer and more selective compounds. One novel avenue has involved the development of benzodiazepine receptor partial agonists, agents which have lower intrinsic efficacy than full agonists and which require higher receptor occupancy for a given effect. It has been suggested that this profile may be sufficient to maintain anti-anxiety activity but insufficient to induce adverse responses (Haefely et al., 1990). Bretazenil (Ro16-6028; Hoffmann-la Roche) is a benzodiazepine receptor partial agonist which has been reported to produce anxioselective effects in several rodent conflict and light/dark exploration tests (Martin et al., 1988; Belzung et al., 1989). In the present study, we have compared the effects of this compound with those of chlordiazepoxide in the murine elevated plus-maze, using recently-developed ethological scoring methods (Rodgers & Cole 1993). Subjects, adult male DBA/2 mice, were randomly allocated to treatment conditions (n=10); saline vehicle, chlordiazepoxide (2.5-15.0 mg/kg), saline/Tween 80 vehicle or bretazenil (5.0-30.0 mg/kg). Compounds were administered i.p. 25-30 minutes prior to testing. Tests, of 5 min duration, were conducted under dim red illumination and recorded on videotape. Behaviours scored included traditional and novel (stretched attend postures, head-dipping, closed arm returns, entry latency, non-exploratory behaviour) anxiety-related measures. Data were analyzed by ANOVA and Dunnett's t-tests.

Although chlordiazepoxide was without significant effect upon total entries or rearing, non-exploratory behaviour increased at higher doses ($X\pm s.e.m.$; saline = 19.9 ± 4.3; 10 mg/kg = 52.8 ± 8.1; 15 mg/kg = 60.3 ± 10.5; $P < 0.025$). At the same doses, percent open entries (24.9 ± 2.7, 45.1 ± 5.6, 46.2 ± 9.2) & percent open time (10.4 ± 1.4, 25.4 ± 5.4, 28.7 ± 10.0) were also increased ($P < 0.025$) while stretch attend postures (18.8 ± 1.5, 11.1 ± 1.2, 10.1 ± 1.0), protected stretch attend (75.7 ± 2.9, 47.2 ± 9.2, 42.6 ± 9.3) and protected head-dipping (72.9 ± 8.0, 29.6 ± 8.0, 35.9 ± 9.5) were reduced ($P < 0.01$). Bretazenil did not impair any measure of general activity; rather it increased % open arm entries (max saline-drug diff, 25.8 ± 4.1 vs 49.6 ± 3.7) and % open time (10.2 ± 3.1 vs 30.9 ± 6.0) across the entire dose range tested ($P < 0.05$ -$P < 0.01$). These effects were accompanied by significant reductions ($P < 0.05$ - $P < 0.01$) in the novel anxiety measures of protected stretch attend (84.1 ± 3.2 vs 45.3 ± 4.7) and protected head-dipping (74.7 ± 8.8, vs 48.0 ± 8.8). Profile comparisons for the dose ranges tested support the anxioselective effects of bretazenil.

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108P DILTIAZEM DISPLAYS A BIPHASIC EFFECT ON *IN VIVO* BINDING OF [^{3}H]-NITRENDIPINE

Watson W.P. and Little H.J., Department of Pharmacology, Medical School, University Walk, Bristol, BS8 1TD

Dihydropyridine calcium channel antagonists, e.g. nitrendipine, are anti-convulsant and potentiate the acute actions of ethanol (Meyer et al., 1987; Dolin and Little, 1986). In contrast, the benzothiazepine calcium channel antagonist, diltiazem, can display both pro- and anti-convulsive activity and enhanced ethanol withdrawal hyperexcitability (Watson and Little, 1992 a,b). Increases in dihydropyridine binding site number are associated with withdrawal from long-term ethanol treatment (Dolin et al., 1987) and diltiazem increases dihydropyridine binding in vitro (Yamamura et al., 1982). We now examine the binding of nitrendipine in vivo following pretreatment with diltiazem.

Groups of 8-10 male TO mice (30-35g) were used. Diltiazem, dissolved in saline, was given i.p. In vivo binding was measured with 4 µCi of [3H]-nitrendipine via the tail-vein in 0.1ml 20% ethanol (Supavilai and Karobath; 1984). At either 1 or 15 min after injection, the brains were removed and homogenised to 20 mg/ml in ice-cold 50mM pH7.4 Tris-buffer. The radioactivity in 1 ml of crude homogenate provided a measure of total activity. The activity on filter papers after 1 ml homogenate was filtered provided the membrane activity. Non-specific binding was determined by incubating 1 ml crude homogenate for 1h at 22°C with 10 µM nimodipine; this was then filtered and counted. All measurements were made in triplicate. Specific binding was the number of counts in filtered homogenate minus that for non-specific binding. To correct for variations in weight or metabolism, this was expressed as a percentage of the total activity. Mice were pretreated with saline or diltiazem, 100 mg/kg, 30 min before injection of [3H]-nitrendipine.

In vivo specific binding in the controls was lower at 15 min than at 1 min. At 1 min the binding was significantly reduced by diltiazem, whereas at 15 min it was greatly increased by this drug. Results (as % of total activity) were:- at 1 min: saline 24.3 ± 1.8, diltiazem 6.1* ± 1.2; at 15 min: saline 8.1 ± 1.9, diltiazem 12.9* ± 1.9 (*P<0.05, Student's T-test).

Binding of nitrendipine to calcium channels is voltage dependent (Bean, 1989). It is possible that the control binding was affected by the unavoidable use of ethanol vehicle (ethanol dose = 0.5 g/kg). The biphasic action of diltiazem may relate to the effects of diltiazem in some models of convulsive states, if a delayed action of diltiazem depolarises neuronal cells and increases susceptibility to seizures. When the initial effects of diltiazem are studied in models of convulsions, therefore, the reduction of the number of active calcium channels may cause the observed anti-convulsive effects of this drug, while later effects of diltiazem (eg increased convulsion incidence and withdrawal hyperexcitability) may relate to the increased binding, indicating increased calcium channel activity.

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109P EFFECTS OF CHRONIC TREATMENT WITH ETHANOL, WITH AND WITHOUT A DIHYDROPYRIDINE CALCIUM CHANNEL ANTAGONIST, ON CALCIUM-INDUCED LONG TERM POTENTIATION

T. L. Ripley and H. J. Little, Department of Pharmacology, The Medical School, University Walk, Bristol BS8 1TD

Prolonged ethanol treatment produced a decreases in long term potentiation, LTP (Durand and Carlen, 1984), a form of synaptic plasticity which has been suggested to be associated with memory. We have shown that concurrent chronic administration of the dihydropyridine calcium channel antagonist, nitrendipine, prevented the development of ethanol tolerance (Dolin and Little, 1989), ethanol withdrawal signs (Whittington et al, 1990) and the effects of chronic ethanol treatment on tetanic LTP.We have now examined the effects of such treatment on LTP produced by a transient increase in calcium concentration in the bathing medium.

Male C57 mice (35-40g) received either 24% ethanol v/v (10-14 g/kg/day), 24% ethanol plus nitrendipine 1.77 mM, or tap water, for 18 weeks. The intake of ethanol was not significantly different for the former two groups; nitrendipine was removed from the drinking solution 24h before ethanol withdrawal. Each treatment group consisted of 5-7 mice. Hippocampal slices were prepared immediately on withdrawal from ethanol and extracellular recordings made from area CA1, with stimulation of the Schaffer collateral commissural pathway (stimulus intensity for half-maximal response, duration 50 μsec). At 4h from ethanol withdrawal (i.e. from slice preparation) the stimulators were turned off for 40 min and the extracellular calcium concentration raised from 2 to 4 mM for the first 7 min. One group of slices from control animals was kept in 2mM calcium to control for turning off the stimulators. Single (T1) and multiple (T2) population spike thresholds (stimulation cut-off = 1000 μA) were measured immediately before and 120 min after the increase in calcium. The % change in population spike height (% change P.S.) from immediately before the increased calcium to 120 min after was calculated, normalised to the first measurement. Maintenance of potentiation (Main.P.) was measured as the % decrease in population spike height from when the stimulators were turned on again until end of recording (from 40 min to 120 min after increase in calcium).

Table 1	Control	Ethanol	Ethanol + Nitrendipine	2mM Ca_{2+} only
Change T1, μA	140 ± 10 → 130 ± 11	137 ± 10 → 129 ± 8	147 ± 7 → 137 ± 7	139 ± 11 → 134 ± 14
Change T2, μA	1000 ± 0 → 1000 ± 0	894 ± 106 → 674 ± 132	1000 ± 0 → 683 ± 145	1000 ± 0 → 920 ± 80
% change P.S.	40.1 ± 1.5	38.8 ± 6.9	27.1 ± 4.5 *	16.7 ± 5.0
Main.P. (% dec.)	0.1 ± 4.4	2.9 ± 4.4	8.7 ± 2.5	20.0 ± 6.7

Values = mean ± s.e.m., Student's t-test - * $p<0.05$ compared with controls

Chronic ethanol treatment did not alter the extent or maintainence of the calcium-induced LTP of the population spike, up to 2h from induction. Chronic ethanol plus nitrendipine, however, decreased the population spike height measured at the end of the recording period, indicating some decrease in maintainence of the potentiation. The results therefore showed a different pattern from the effect of chronic ethanol and nitrendipine administration on tetanically-induced LTP (Ripley and Little, this meeting). The neuronal mechanisms underlying these two types of LTP therefore appear to be affected differently by chronic ethanol treatment and by nitrendipine.

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110P METHADONE IS INCORPORATED INTO HAIR BY SATURABLE PROCESSES

S.J. Green & J.F. Wilson, Department of Pharmacology & Therapeutics, University of Wales College of Medicine, Cardiff CF4 4XN

It has been proposed that drug incorporation into hair occurs from blood into the germinal hair cells with the drug becoming tightly bound during subsequent keratogenesis (Baumgartner *et al.*, 1989). Routes through sebum and sweat have also been suggested. We have investigated the relationships between oral dose, plasma concentration and hair drug content for methadone in the albino rat.

Methadone was administered in the drinking water at concentrations of zero, 0.05, 0.1, 0.15, 0.2 and 0.25mg/ml to groups of six male Wistar rats final body weight 239±12g (±s.e.mean). Water intake was monitored daily. After one week of treatment, hair was shaved from the back and flanks. The hair was allowed to regrow under continuing methadone treatment for 5 weeks before collection of trunk blood and of hair samples from the areas previously shaved. Ten mg samples of hair were digested overnight in 1ml of 1M NaOH at 45°C. The digest was extracted with 5ml of a 9:1 chloroform:isopropyl alcohol mixture and the dried organic extract reconstituted in 1ml phosphate buffer. The methadone content of the hair extracts and blood samples was measured by radioimmunoassay (Coat-a-Count, DPC, Llanberis, UK). Recovery from hair was estimated to be >83% by the method of standard addition.

Oral intake of methadone increased linearly with water content from 0 to 32±1mg/kg/day (Figure 1A). Plasma methadone concentrations increased with increasing oral intake across the dose range studied reaching a mean of 53±17ng/ml with the highest dose (Figure 1B). Methadone concentration in hair increased significantly ($p<0.05$, one-way analysis of variance) over the first two dose levels to 6.8±2.4ng/mg (Figure 1C). At the higher dose levels, hair methadone concentration did not rise further ($p>0.05$).

We conclude that methadone is incorporated into hair in the rat by capacity limited processes which become saturated at plasma concentrations greater than some 30ng/ml.

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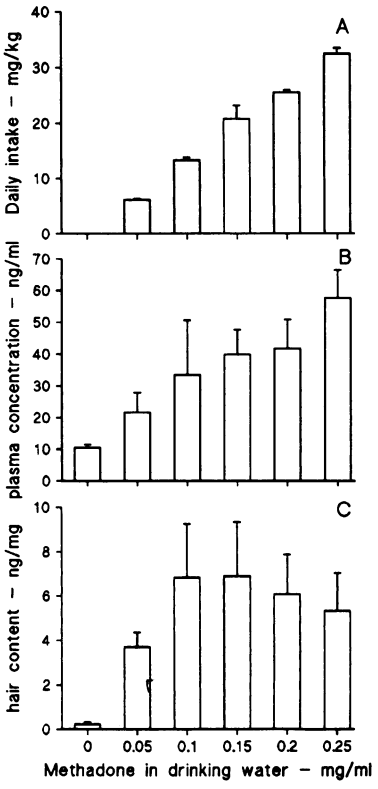

111P α_2-ADRENOCEPTOR AND 5-HT$_1$-MEDIATED RESPONSES IN BOVINE PULMONARY ARTERIES: THE EFFECT OF VASCULAR TONE AND FORSKOLIN

R.A. Clayton, A.G.B. Templeton & M.R. MacLean. Inst. of Physiology, University of Glasgow, Glasgow G12 8QQ.

Previous studies have shown, in the perfused rat tail vascular bed that responses to the α_2-adrenoceptor agonist UK 14304 are potentiated in the presence of tone (Templeton *et al.*, 1989; MacLean & McGrath, 1990). Both the 5-hydroxytryptamine$_{1D}$-receptor agonist, sumatriptan (SUM) and UK 14304 are thought to mediate their contractile effects through decreasing intra-cellular (i.c.) cAMP levels. Here we investigate the effect of increased vascular tone and activation of adenylate cyclase (to increase i.c. cAMP levels) with forskolin on responses to sumatriptan and UK 14304 in the bovine pulmonary artery.

Rings of bovine pulmonary artery (3-5mm i.d.) were set up in Krebs-filled organ baths under 1.5g tension and bubbled with 95%O_2/5%CO_2 at 37°C. Cumulative concentration-response curves (CCRs) were constructed to SUM and UK 14304 (3nM-10μM). After washing, these CCRs were repeated in the presence of tone induced by 10nM U46619 (thromboxanemimetic). After washing and re-establishing the increase in tone, the CCRs were repeated in the presence of forskolin (1nM, 10nM & 100nM). Relevant time-controls were also established. Forskolin caused a vasorelaxation and so to control for this, CCRs were also repeated in the presence of sodium nitroprusside (1nM, 10nM & 100nM, SNP) which is a vasodilator which acts through elevating i.c. cGMP levels.

Only 40% of the vessels tested contracted to SUM but in the presence of tone, 100% responded. UK 14304 did not induce a significant vasoconstriction in the absence of tone. With tone present, both SUM and UK 14304 induced vasoconstriction in a concentration-dependent manner. The effect of vascular tone alone or tone plus forskolin or SNP on maximum responses to the agonists is shown in Table 1.

Table 1. Effect of vascular tone, forskolin and sodium nitroprusside on maximum responses to UK 14304 and SUM in bovine pulmonary artery rings.

Pharmacological intervention	**% change in vascular tone**	**% change in response to UK 14304**	**% change in response to sumatriptan**
Increased tone		+400-4000 ***	+100-1000 ***
Forskolin (1nM)	-3 ± 2	+15 ± 15	+35 ± 15 *
Forskolin (10nM)	-29 ± 5	+37 ± 16 *	+32 ± 14 *
Forskolin (100nM)	-85 ± 4	+72 ± 26 **	+126 ± 15 ***
SNP (1nM)	-5 ± 3	-14 ± 16	+16 ± 11
SNP (10nM)	-35 ± 7	-56 ± 5 ***	+4 ± 12
SNP (100nM)	-94 ± 3	-68 ± 10 ***	-67 ± 7.5 ***

Significant difference from control responses was assessed using a paired Student's t-test. *P<0.05, **P<0.01, ***P<0.001

The results show that raising vascular tone facilitates responses to both UK 14304 and SUM. The maximum responses achieved were, however, only approximately 30% of the responses achieved by a maximal concentration of, for example, U46619. Responses to the agonists overcame the forskolin-induced relaxations so maximum responses to both UK 14304 and SUM were potentiated. SNP induced reductions in vascular tone comparable to those induced by forskolin. In the presence of SNP-induced relaxations, responses to UK 14304 and SUM were reduced. These results support the suggestion that these agonists achieve vasoconstriction by decreasing i.c. cAMP levels as provision of increased i.c. levels of cAMP by forskolin can increase the extent to which these agonists induce vasoconstriction.

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112P COMPARISON OF RABBIT NEONATE AND ADULT PULMONARY ARTERIAL RESPONSES TO 5-HYDROXYTRYPTAMINE

M.R. MacLean, K.M. McCulloch, A.G.B. Templeton and M. Baird. Inst. of Physiology, University of Glasgow, Glasgow G12 8QQ.

Treatment of persistent pulmonary hypertension of the neonate (PPHN) is compromised by the absence of specific pulmonary vasodilators (Fox & Duara, 1983). 5-hydroxytryptamine (5-HT) was thought to constrict pulmonary arteries (PAs) primarily through 5-HT$_2$-receptors (Frenken & Kaumann, 1984). We have shown that the 5-HT$_1$-receptor agonist, sumatriptan, constricts bovine PAs but not mesenteric arteries (Templeton *et al.*, 1993a) and is equipotent with 5-HT in human pulmonary arteries (Templeton *et al.*, 1993b). 5-HT may play a vasoconstrictor role in secondary pulmonary hypertension (eg. Seibold *et al.*, 1987). Here we describe a preliminary study into the sensitivity to 5-HT of rabbit neonate PAs compared to adult PAs. We also investigated the effect of endothelial removal on 5-HT responses in the adult PAs.

1 day old, 4 day old and adult rabbits were killed with sodium pentobarbitone. The main PA and its first left branch were dissected out from neonates and adults and suspended, as ring preparations (under 0.6g and 2g tension respectively) in Krebs (PO_2 of 45-50 mmHg) in 5 ml organ baths. Concentration-response curves for 5-HT (1nM - 300μM) were constructed. The endothelium was removed by rubbing of the intimal surface of the PA rings (endothelium-dependent relaxations were assessed by adding substance P (100pM-100nM) to 5-HT pre-constricted vessels). Results are shown in Tables 1a and b.

Table 1. Developmental changes in rabbit PA sensitivity to 5-HT and the effect of endothelium removal. **A.** Main PA. **B.** First left PA branch.

Age	**A.**	EC_{50} (-log M)	n	**B.**	EC_{50} (-log M)	n
1 day		7.1 ± 0.1**	6		7.1 ± 0.03***	4
4 day		6.1 ± 0.2**	5		7.4 ± 0.2***	8
Adult (control: endothelium intact)		5.4 ± 0.1	5		5.8 ± 0.1	5
Adult (endothelium rubbed)		6.0 ± 0.1**	5		6.2 ± 0.1*	5

Significant differences from adult control: *P<0.05, **P<0.01, ***P<0.001 (Student's unpaired t-test).

Endothelium removal also increased the maximum responses of the adult main PA from 39 ± 8 (% response to 50 mM KCl) to 87 ± 18% (P<0.05) and those of the PA branch from 29.6 ± 12.6% to 77.4 ± 10% (P<0.05). Endothelium-dependent relaxations to substance P were observed consistently only in the control adult rabbit vessels and were absent in those with rubbed endothelium.

The results show that neonate PAs are extremely sensitive to 5-HT compared with adult PAs and hence appropriate 5-HT antagonists may prove useful in PPHN. Endothelium removal also increased the sensitivity and maximum responses of adult PAs to 5-HT. Liu *et al.*, (1992) showed that, in porcine PAs, endothelium-dependent vasodilator responses were not observed until 3-10 days after birth. This and the results described here suggest that neonate PA supersensitivity to 5-HT may, in part, be the result of the absence of a functional endothelium.

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113P SENSITIVITY OF HUMAN, BOVINE AND RABBIT PULMONARY ARTERIES TO SUMATRIPTAN: COMPARISON WITH 5-HT

A.G. B. Templeton, R.A. Clayton, P.D. McIntyre*, S.W. Hillis*, A.J. Peacock† & M.R. MacLean. Institute of Physiology & Depts of Cardiology* & Respiratory Medicine†, University of Glasgow, Glasgow G12 8QQ.

Sumatriptan (GR43175, SUM) is a 5-HT_1-like agonist which is typically several orders of magnitude less potent than 5-HT in veins or systemic arteries (e.g. Humphrey *et al.*, 1988; Parsons *et al.*, 1989). We have previously shown that SUM contracts bovine pulmonary arteries (PAs) but not bovine systemic mesenteric arteries (Templeton *et al.*, 1993) and may be a more selective agonist for the pulmonary circulation. Here we compare vasoconstrictor responses to 5-HT and SUM in human, bovine and rabbit PAs.

Ring preparations of human intra-PAs (2-3mm i.d. [healthy sections of post-operative bronchial carcinoma tissue]), bovine intra-PAs (3-5mm i.d. [from lung supplied by local abbatoir]) and rabbit extrapulmonary arteries (extra-PAs) (3-6mm i.d.) were set up in Krebs-filled organ baths under 2g tension bubbled with 5% O_2, 6% CO_2 (extra-PAs) and 95%O_2,5%CO_2 (intra-PAs). Concentration-response curves to SUM and 5-HT (10nM-10μM) were constructed. The results are shown in Table 1. Only 40% of bovine vessels responded to SUM and it is the data from these that is shown. However, responses to SUM are potentiated in the presence of vascular tone (Clayton *et al.*, 1993) and under these conditions 100% of the preparations responded to SUM.

Table 1. EC_{50} values (-log M) for 5-HT and sumatriptan as well as the maximum response to SUM as a % of the maximum response to 5-HT in the same preparation. n/n = number of ring preparations/number of individuals

Species	Agonist	n/n	EC_{50} values (-log M)	Maximum response to SUM (% of max to 5-HT)
Human	5-HT	13/9	6.52 ± 0.11	
Human	sumatriptan	19/9	6.35 ± 0.09	59.7 ± 5.3%
Bovine	5-HT	10/10	6.49 ± 0.11***	
Bovine	sumatriptan	14/14	5.56 ± 0.09	59.2 ± 4.5%
Rabbit	5-HT	5/5	5.78 ± 0.14***	
Rabbit	sumatriptan	5/5	no response	0%

*Significant differences between EC_{50} values for sumatriptan and 5-HT were assessed using the unpaired Student's t-test.*** $P<0.001$*

The results show that bovine PAs are 10 x more sensitive to 5-HT than SUM whilst rabbit PAs are insensitive to SUM. Human PAs, however, are as sensitive to SUM as they are to 5-HT although SUM does not induce as great a maximum response as 5-HT. This indicates that 5-HT_1-receptors are present in human PAs and may, therefore, contribute to the physiological responses to 5-HT. The 5HT_2-receptor antagonist ketanserin is effective in the treatment of secondary pulmonary hypertension but also has a systemic hypotensive effect (Reneman & Starre, 1990). The results presented here and in our previous studies suggest that a selective 5-HT_1 antagonist may be a selective pulmonary vasodilator and effective in the treatment of pulmonary hypertension.

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114P SPASMOLYTIC ACTIVITY OF HIMBADINE IN ISOLATED GUINEA-PIG ILEUM AND RABBIT JEJUNUM

A.H. Gilani, S.A.Saeed and A. Suria, Department of Pharmacology, The Aga Khan University, Stadium Road, Karachi-74800, Pakistan.

Himbadine and himandrine are alkaloids, isolated from bark of the *Galbulimima baccata* (Ritchie & Taylor, 1967). Earlier studies with himandrine have shown that it produces hypotension and bradycardia in anaesthetized cats. It also produces spasmolytic activity in isolated smooth muscle preparations (Cobbin and Thorp, 1957). However, studies on himbadine are scarce. In the present investigation, we describe effects of himbadine on isolated guinea-pig ileum and rabbit jejunum.

Segments of guinea-pig ileum (n=8) and rabbit jejunum (2 cm; n=8) were mounted in a tissue bath of 20 ml capacity filled with Tyrode's and Krebs' solutions, respectively. The temperature of the tissue bath was maintained at 37 °C and aerated with a mixture of 5% carbon dioxide and 95% oxygen. The tissues were allowed to equilibrate under 1 g resting tension for 30 min before administration of any drug, and isotonic responses were recorded on a Bioscience MD4 oscillograph.

In guinea-pig ileum, himbadine (0.1 $mg.ml^{-1}$) inhibited acetylcholine- or histamine-induced contractions to a similar extent, indicating non-specific spasmolytic activity. Himbadine at concentrations of 0.03-0.3 $mg.ml^{-1}$ inhibited spontaneous movements of the rabbit jejunum in a concentration-dependent manner. K^+ (40 mM) caused a sustained tonic contraction, and addition of himbadine to the tissue bath produced inhibition of spontaneous contraction in a concentration-dependent manner. Replacement of the bathing fluid with Ca^{++}-free Krebs' solution abolished spontaneous movements of rabbit jejunum, which were restored on addition of Ca^{++} (30 mM). However, in preparations pretreated with himbadine (0.3 $mg.ml^{-1}$), the addition of Ca^{++} (up to 50 mM) failed to restore spontaneous contractions of the rabbit jejunum.

The contraction of smooth muscles by high K^+ is dependent upon ingress of Ca^{++} into the cells through voltage-dependent calcium channels (VDCs; Bolton, 1979). One mechanism responsible for the inhibition of high K^+-induced contraction of the rabbit jejunum by himbadine may therefore be a reduction in Ca^{++} entry through VDCs.

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115P MAGNESIUM AS AN INHIBITOR OF SMOOTH MUSCLE CONTRACTION IN THE HUMAN LARGE BOWEL

F.J. Hemmings, R.H. Grace[§] and N.J. Birch, Biomedical Research Laboratory, School of Health Sciences, University of Wolverhampton, Wolverhampton, WV1 1DJ and [§] Department of Gastroenterology, Royal and Wolverhampton Hospitals.

In the rodent, magnesium has been shown to have inhibitory effects on gut motility (Altura *et al.*, 1982; Edbury *et al.*, 1991) causing contraction responses to a number of small intestinal preparations in the presence of this ion. Recent work from this laboratory has demonstrated that magnesium (23.8 mM) reduced the maximal contraction responses to carbachol by 69.9% in the jejunum, 65.3 % in the ileum, 11.7% in the colon and 6.3% in the stomach fundus (Edbury *et al.*, 1991). We have now investigated the potential inhibitory properties of magnesium ions in the human large bowel.

Using the isolated organ bath technique, we have investigated the effects of increasing buffer magnesium concentrations from 1.19 mM to 23.8 mM on carbachol-induced contractions of the human large bowel resected for carcinomas. Paired dose/response curves were performed on seemingly "healthy" strips of bowel taken from as far away from the carcinoma as possible. Enhanced magnesium content of the buffer produced 71.9 $\pm$ 4.13% inhibition ($n=10$ from 4 specimens; $p \leq 0.005$) of the carbachol-induced contractions.

Further investigations to determine the mechanism of magnesium inhibition in this system were performed by producing carbachol-induced contractions in calcium free conditions and adding cumulative doses of $CaCl_2$ in increments of 2.5 mM to a total of 12.5 mM. This was performed at "control" (1.19 mM) and enhanced (23.8 mM) magnesium concentrations. In "control" magnesium conditions, carbachol produced no contractions in calcium free buffer but on the addition of cumulative doses of calcium (2.5 mM), up to a final concentration of 7.5 mM, responses returned to almost 98.6 $\pm$ 23.9% of those produced in control calcium concentrations. However, in the presence of an increased concentration of magnesium ions (23.8 mM), addition of calcium, up to a final concentration of 12.5 mM, produced only 16.40 $\pm$ 12.5% of the response produced in control conditions.

Stimulation of muscle contraction by carbachol is triggered by increased influx of calcium into the cell from multiple sources (Brading and Sneddon, 1982). The inhibitory effect of magnesium may therefore be due to competition between calcium and magnesium for binding sites or carriers on the membrane (Prasad *et al.*, 1973). Our results support this and we conclude that, in the human large bowel, magnesium is a potent inhibitor of carbachol-induced contractions and acts via inhibition of calcium ion entry.

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116P MUSCARINIC AND β-ADRENOCEPTOR BINDING SITES IN THE NORMAL AND UNSTABLE HUMAN BLADDER

T.E. Beavington, J.F.B. Morrison, A.S. Robertson[1] & D.E. Neal[1], Dept. of Physiology, University of Leeds, Leeds, LS2 9NQ and [1]Dept. of Surgery, Freeman Hospital, Freeman Road, High Heaton, Newcastle-upon-Tyne, NE7 7DN (Introduced by I.E. Hughes)

Lower urinary tract symptoms are commonly due to bladder instability, a urodynamic diagnosis made when the detrusor contracts, either spontaneously or on provocation, during the filling phase of the cystometrogram, resulting in a pressure rise exceeding $15cmH_2O$. One current theory suggests that denervation of the bladder smooth muscle results in it developing spontaneous activity (Sethia *et al.*, 1990); Gunasena *et al.* (1993) demonstrated a two-fold increase in muscarinic receptors in the denervated rat bladder. In the present study radioligand binding techniques were used to investigate any changes in muscarinic and beta-adrenoceptor binding sites in the unstable human bladder.

Tissues were obtained from ten control patients and from five patients whose bladders were classed as unstable on the basis of urodynamic investigations. In all cases samples were removed from the body of the bladder and included no trigonal or bladder neck tissue. Once removed, the bladder specimens were snap frozen in Arcton-12, cooled to -196°C with liquid nitrogen and then stored at -80°C until required. 20μm sections were cut on a cryostat at -18°C and thaw-mounted onto poly-l-lysine-subbed slides. The slides were randomised and a few representative slides from each sample were used for protein estimations. Sections were incubated with increasing concentrations of the muscarinic antagonist l-quinuclidinyl [phenyl-4-^{3}H] benzilate (0.2-8nM ^{3}H-QNB, specific activity 47-50 Ci mmol^{-1}) for 90 minutes at 25°C. Non-specific binding was determined in the presence of 10^{-4}M atropine. A similar technique was employed to measure beta-adrenoceptor binding sites using the highly specific beta-adrenoceptor antagonist [^{125}I]iodocyanopindolol (^{125}I-CYP) as ligand. This time slides were incubated with increasing concentrations of ^{125}I-CYP (2.5-75pM ^{125}I-CYP, specific activity 2000 Ci mmol^{-1}) for 2 hours at 37°C. Non-specific binding was determined in the presence of 2mM isoprenaline.

Scatchard analysis of ^{3}H-QNB binding to bladder sections revealed a significant ($p < 0.001$) increase in muscarinic binding sites in the unstable bladders ($B_{max}=452\pm32$ fmol/mg protein, $n=5$) compared to the controls ($B_{max}=238\pm23$ fmol/mg protein, $n=10$) [mean$\pm$s.e.mean]. Specific ^{3}H-QNB binding was saturable and of high affinity and the mean equilibrium dissociation constants for the control and unstable bladders were 1.4$\pm$0.2nM and 1.3$\pm$0.5nM respectively. There was no significant difference ($p > 0.5$) in the density of beta-adrenoceptor binding sites in 6 normal ($B_{max}=23.7\pm5.2$ fmol/mg protein) and 3 unstable bladders ($B_{max}=21.6\pm8.2$ fmol/mg protein). ^{125}I-CYP binding was saturable and of high affinity and the dissociation constants for the control and unstable bladders were 9.6$\pm$2.3 pM and 9.3$\pm$2.1 pM respectively. In conclusion there appears to be a two-fold increase in muscarinic binding sites in the unstable bladder and no change in beta-adrenoceptor binding density. In addition, there appears to be no change in the affinity of either binding site.

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117P CARDIOTONIC ACTIONS OF THE NOVEL VASODILATOR, FLOSEQUINAN, AND ITS METABOLITE, BTS 53554, IN ISOLATED VENTRICULAR CARDIOMYOCYTES

E.J. Kelso, B.J. McDermott & B. Silke, Department of Therapeutics and Pharmacology, The Queen's University of Belfast, Whitla Medical Building, 97 Lisburn Road, Belfast. BT9 7BL.

Although much attention has been directed over the past decade to the development of new positive inotropic agents, such as phosphodiesterase inhibitors, for the management of congestive heart failure (CHF), the results have been discouraging (Packer *et al.*, 1991). Flosequinan is a novel vasodilator which produces haemodynamic improvement when given to patients with CHF (Corin *et al.*, 1991). This study was designed to assess the direct inotropic effects of flosequinan and its metabolite, BTS 53554 (7-fluoro-1-methyl-3-methylsulphonyl-4-quinolone), in ventricular cardiomyocytes isolated from adult Sprague Dawley rats (200-250g) and from New Zealand White rabbits (2-2.5kg). Contractile function, as measured by mechanical cell shortening (dL), and biochemical systems involving cyclic AMP, were investigated.

Flosequinan and its metabolite, BTS 53554, produced positive contractile effects which were concentration-dependent over the range, 10^{-5}-10^{-3}M, in experiments in both rat (n=5) and rabbit (n=3) cardiomyocytes. dL values for mean basal levels were 7.46±0.17% and 7.44±0.11% in rat and rabbit cells, respectively. In rat cardiomyocytes, flosequinan and BTS 53554, at maximum concentrations used (10^{-3}M), increased contractile amplitudes by 28.8±4% and 44.7±13%, respectively, over basal values. The opposite trend was found in rabbit cardiomyocytes; the contractile amplitudes produced by flosequinan and BTS 53554, at concentrations of 10^{-3}M, were 31±2% and 21±3%, respectively. In rat cardiomyocytes, maximum stimulation was reached at a concentration of 10^{-3}M by flosequinan but not by BTS 53554; the concentration of flosequinan producing 50% of maximum contractile amplitude (EC_{50}) was 5.8±0.6 x 10^{-5}M. On the other hand, in rabbit cardiomyocytes, maximum stimulation was reached at a concentration of 10^{-3}M by BTS 53554 (EC_{50} value = 6.07±0.6 x 10^{-5}M), but not by flosequinan. The interaction of flosequinan or the metabolite with other PDE inhibitors was studied in experiments (n=7) in rat cardiomyocytes. Contractile amplitudes were not significantly different in the presence of equimolar concentrations ($3x10^{-4}$M) of Ro 20-1724, flosequinan or BTS 53554 alone (15±6%, 18±4% and 32±10%, respectively, greater than the mean basal dL value of 7.38±0.12%). However, the combinations of Ro 20-1724 with flosequinan and of Ro 20-1724 with BTS 53554 produced synergistic responses, 71±10% and 72±14%, respectively, greater than the mean basal dL value ($P<0.05$). In contrast, the combinations of either flosequinan or BTS 53554, with IBMX or sulmazole, did not produce any further increase in contractile amplitude. Also, neither flosequinan or BTS 53554 produced any detectable increase in accumulation of cyclic AMP, whereas significant increases were noted by Ro 20-1724, IBMX and sulmazole, in rat cardiomyocytes.

In summary, flosequinan and BTS 53554 produced concentration-dependent positive contractile effects in rat and rabbit cardiomyocytes. Less than additive effects between the non-selective PDE inhibitor, IBMX, or the selective PDE III inhibitor, sulmazole, with either flosequinan or BTS 53554, in addition to a potentiated response of these drugs in combination with the selective PDE IV inhibitor, Ro 20-1724, indicates that flosequinan and BTS 53554 selectively inhibit the PDE III isoenzyme in these heart muscle cells, but only at concentrations higher than obtained clinically.

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118P EFFECT OF DIGOXIN-SPECIFIC FAB FRAGMENTS ON RENAL FUNCTION IN THE RAT

R.J. Moran, A.D. Struthers & D.S Hewick, Dept. Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee DD1 9SY

Digoxin-specific antibody fragments (DSFab) are used clinically in the treatment of severe digitalis intoxication. They bind the free drug and then the Fab-hapten complex is subsequently filtered through the glomerulus before urinary excretion. Recently it has been reported that DSFab may adversely affect renal function in the rabbit (Timsina & Hewick, 1992). However, since this study lacked a separate control group of animals and since the adverse effect had not been observed in an earlier study in rats (Pentel *et al*, 1988), we proposed to further investigate the influence of DSFab on renal function using the rat as an experimental model.

Rats were given an intravenous bolus dose of either DSFab (2 mg kg^{-1}) or saline. Blood was sampled from the tail vein and urine collected via a metabolism cage before and after injection. Creatinine in plasma and urine, urinary Na^+ and DSFab were measured as described in Timsina and Hewick (1992). Means ± s.e. mean are given.

After DSFab, creatinine clearance was reduced from 6.82 ± 1.62 to 4.43 ± 1.19 ml kg^{-1} $min.^{-1}$ ($P<0.05$), there was also a non-significant trend towards decreased urine volume. Plasma and urine creatinine concentration and urinary Na^+ excretion were not altered significantly by the treatment. In the control group, no significant effect was observed on any parameter after injection of saline and blood sampling. DSFab had an elimination half-life of 178 ± 6 min., an apparent volume of distribution of 106 ± 13 ml kg^{-1} and a plasma clearance of 0.42 ± 0.05 ml kg^{-1} $min.^{-1}$. Within 2 h after injection, 2.2 ± 0.5% of the administered dose of DSFab was measured in the urine.

Since creatinine clearance approximates to glomerular filtration rate (GFR), it appears that 2 mg kg^{-1} DSFab (equivalent to a sub-therapeutic dose) causes a reduction in GFR of about 35% and is in agreement with the study of Timsina & Hewick (1992) in rabbits, but not that of Pental *et al* (1988) in rats in which very large doses of DSFab were given. In patients undergoing digitalis therapy, a degree of renal impairment is common and it is possible that this may be exacerbated by treatment with DSFab.

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119P EFFECT OF CHRONICALLY HIGH INSULIN LEVELS ON POTASSIUM CHANNELS IN RAT VENTRICULAR MYOCYTES

A.C.Dawson, M.Gwilt[1] & D.Wray, Department of Pharmacology, University of Leeds, Leeds LS2 9JT and [1] ZENECA Pharmaceutics, Alderley Park, Macclesfield SK10 4TG

Chronically high insulin levels may underlie many conditions such as hypertension, atherosclerotic heart disease and heart failure (Prichard et al., 1992). The mechanism by which insulin produces these effects is largely unknown; however effects on ion transport mechanisms (such as the Na^+-H^+exchanger, the Na^+ -K^+ ATPase and Ca^{2+} ATPase pumps) may be implicated (DeFronzo,1992). In this study we investigated the effects of chronically high insulin levels on potassium channels in the heart. For this, we used obese Zucker rats (which have high insulin levels), and compared our measurements of potassium currents with those in (normal) Wistar rats. Ventricular myocytes were enzymatically dissociated from male rat hearts based on the method described by Isenberg and Klockner (1982). Currents were measured in the whole cell patch clamp configuration using an Axopatch 1-C. All experiments were carried out at 22-24°C in a HEPES buffered Tyrode solution; 3mM cobalt was present to block any calcium currents. Cells were held at a potential of -80mV, and test pulses were elicited every 10 seconds with duration 200ms. In addition to fast inward sodium spikes, currents had a transient outward component (I_{Kto}) and a sustained component (I_{K1} for hyperpolarising pulses) . The amplitudes of the sustained current at the end of the 200ms pulse and of the transient outward component were measured. The sustained current in the cells from Zucker rats was greater than the corresponding current from Wistar rats. This difference was statistically significant (Student's t test, $P<0.05$) at test potentials of +20mV to +40mV (e.g. at +40mV, currents were 1.31±0.13 nA, n=13 cells for Zucker rats, and 1.025±0.083 nA, n=16 for Wistar rats, mean± s.e.mean). The transient outward component was also greater for Zucker rat cells than for Wistar rats (e.g. at a test potential of +40mV, currents were 1.09 ± 0.21 nA, n=13 for Zucker rats, and 0.77±0.12 nA, n=16 for Wistar rats). The extent of the block of the sustained and transient components by 250μM 4-aminopyridine (4-AP) was tested. There were no significant differences between the extent of block by 4-AP on the transient component in Zucker and Wistar rats. On the other hand, the extent of block of the sustained component by 4-AP in Zucker rats was greater(e.g. at a test potential of +40mV, the sustained component was blocked by 30 ± 13 %, n=5 cells for Zucker rats, and by 7.4 ± 9.3 %, n=3 for Wistar rats). These increases in potassium currents and changes in the extent of block by 4-AP in Zucker rats could be interpreted to imply that chronically high insulin levels may cause upregulation of potassium channels in the heart.

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120P LACK OF EFFECT OF CLOFILIUM ON REPOLARISATION IN THE RAT ISOLATED HEART CONFIRMS THAT RAT VENTRICLE IS DEVOID OF FUNCTIONAL DELAYED RECTIFIER POTASSIUM CURRENT

Siân A. Rees & Michael J. Curtis, Cardiovascular Research Laboratories, Pharmacology Group, Division of Biomedical Sciences, King's College, Manresa Road, London SW3 6LX.

Clofilium is termed a class III antiarrhythmic because of its ability to prolong cardiac action potential duration (Steinberg & Molloy, 1979). Its prolongs repolarisation primarily by blockade of the delayed rectifier K+ current (I_K) with no effect on the inwardly rectifying K+ current at concentrations below 100 μM (Arena & Kass, 1988). We have previously presented evidence that rat ventricle is devoid of the rapidly activating component of I_K (I_{Kr}; Rees & Curtis, 1993). In the present study we used clofilium to investigate whether rat ventricle possesses the slowly activating component of I_K (I_{Ks}), since clofilium has been shown to block this component of I_K (Sanguinetti & Jurkiewicz, 1990). Clofilium has also been shown to block the transient outward K+ current in rat, but its onset of action is extremely slow (with block occurring after cells have been incubated with 3 μM clofilium for 3 hours; Castle, 1991).
Isolated rat hearts (n=10/group) were perfused with solution containing (in mM) NaCl 118.5, $NaHCO_3$ 25.0, KCl 3.0, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.4 and glucose 11.1 (pH 7.4, 37°C) for 5 min then hearts were randomised to similar solution containing clofilium (3 μM) or vehicle (water) at time = 0 min. Measurements were taken of QT interval at 100 % repolarisation, RR interval and PR interval at time = -1, +30 and +60 min; *$p<0.05$ versus controls.

Table 1.

	Intervals (msec) measured in control hearts			*Intervals (msec) measured in clofilium hearts*		
Time (min)	QT interval	PR interval	RR interval	QT interval	PR interval	RR interval
-1	107±7	37±2	211±9	102±7	38±1	229±9
+30	155±12	42±2	300±8	163±17	44±3	332±16
+60	157±10	40±2	293±8	186±16	47±3	332±18

Values of each variable tended to increase with time during perfusion in both groups, but there were no significant differences in QT interval, PR interval or RR interval, between groups at any time (Table 1). In conclusion, at concentrations sufficient to block I_{Ks}, in a species deficient in functional I_{Kr}, clofilium was without effect on electrogram intervals, indicating that rat ventricle is deficient in functional I_{Ks}.

(S.A.R. is a recipient of the A.J. Clark Award from the British Pharmacological Society)

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121P ISCHAEMIA-INDUCED AND REPERFUSION-INDUCED ARRHYTHMIAS IN THE ISOLATED RAT HEART ARE SUPPRESSED BY CIMETIDINE WITHOUT HAEMODYNAMIC OR ELECTROCARDIOGRAPHIC ALTERATION

Maggi M. Banning & Michael J. Curtis, Cardiovascular Research Laboratories, Pharmacology Group, Division of Biomedical Sciences, King's College, Manresa Road, London SW3 6LX.

Biochemicals present in the tissue milieu contribute to arrhythmogenesis in ischaemic heart disease, although their relative importance is unclear (Curtis et al., 1993). The role of histamine in initiation of ventricular fibrillation (VF) and the receptor subtype involved in mediating the response are unresolved (Wolff and Levi 1986). We have examined these issues further by use of cimetidine, a specific H_2 receptor antagonist in a model of left regional ischaemia. The hypothesis that histamine, acting via H_2 receptors, functions as a local mediator of VF (Dai 1985), predicts that cimetidine should reduce VF incidence.

Rat hearts (n=12-15 per group) were perfused with solution containing (in mM) CaCl 1.4, NaCl 118.5, $NaHCO_3$ 25.0, $MgSO_4$ 1.4, NaH_2PO_4 1.2, KCl 4.0 and glucose 11.1, gassed with 95% O_2, 5% CO_2, pH 7.4 at 37° C. Hearts were randomised to 0 (control), 0.1, 1.0 or 10 μM cimetidine. After 5 minutes, left regional ischaemia was induced for 30 minutes, whereupon the ischaemic zone was reperfused, with verification by use of disulphine blue dye (Curtis & Hearse 1989). Statistical analysis (Chi^2 or Dunnet's test) was carried out as described previously (Curtis & Hearse, 1989).

Cimetidine reduced the incidence of ischaemia induced VF from 57% in controls to 29%, 17% ($p<0.05$) and 8% ($p<0.05$) at 0.1, 1.0 and 10.0 μM respectively. Corresponding data for reperfusion induced VF were 78%, 57%, 66% and 38% ($p<0.05$) respectively. Control values of coronary flow ($ml.min^{-1}$), e.g. 1 min before the onset of ischaemia (10.0±0.6), 29 min after the onset of ischaemia (4.5±0.1), 1 min after the start of reperfusion (8.6±0.1), and of heart rate ($beats.min^{-1}$) e.g. 1 min before the onset of ischaemia (296±2), and of occluded zone size (36.0±1.0% of total ventricular wt) were not altered by cimetidine at any dose. Cimetidine also had no effect on QT interval at 90% repolarisation, or PR interval; after 10 min of ischaemia QT (msec) and PR (msec) were 85±19 and 30.0±0.8 in controls and 89±16 and 29.2±0.8 with the highest concentration of cimetidine, respectively.

In conclusion, cimetidine possesses concentration dependent antifibrillatory activity in the isolated rat heart during ischaemia and during reperfusion. This activity occurred without manifestation of QT or PR electrogram changes (which would have indicated class I, III, or IV activity) or haemodynamic alteration. The data give support to the hypothesis that histamine (activity mediated via H_2 receptors) may participate as a chemical mediator of arrthythmogenesis in acute myocardial ischaemia and reperfusion.

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122P ZD7288, A NOVEL SELECTIVE SINO-ATRIAL NODE FUNCTION MODULATOR, COMPARED WITH ALINIDINE, ZATEBRADINE AND β-ADRENOCEPTOR ANTAGONISTS IN EXERCISING CONSCIOUS RATS

W. Rouse, P.J. Stafford, I.R. Johnson. VIM Research Department, Zeneca Pharmaceuticals, Macclesfield, UK.

ZENECA ZD7288 (Marshall et al., 1992) was evaluated as a modulator of exercise-induced heart rate (HR) increases in comparison with the clinically-effective zatebradine (Baiker et al., 1991), alinidine (Shanks, 1987), and β-adrenoceptor antagonists atenolol and propranolol.
Seven treadmill-trained beagles were implanted during aseptic thoracotomy under halothane anaesthesia, with ascending aortic flow and LVP transducers, bipolar right atrial and ventricular electrodes, and carotid and jugular catheters. Recovery, for two weeks, was under veterinary supervision.
Minute-meaned beat-by-beat haemodynamic variables were recorded using a hybrid computer system during successive periods of 5 min exercise (6.5 $km.hr^{-1}$, level) with 25 min recovery. Three doses of the drug under study were given cumulatively (iv) between successive pairs of exercise periods.

DRUG	DOSE mg.kg⁻¹ iv	% CONTROL HR RESPONSE	% CONTROL dP_{LV}/dt RESPONSE	DRUG	DOSE mg.kg⁻¹ iv	% CONTROL HR RESPONSE	% CONTROL dP_{LV}/dt RESPONSE
CONTROL	0	106 ± 5	86 ± 5	ALINIDINE	0.01	104 ± 7	86 ± 5
	0	103 ± 4	80 ± 5 *		0.03	96 ± 7	73 ± 7 *
	0	104 ± 3	75 ± 3 **		0.1	89 ± 7	50 ± 7 **
ZD7288	0.1	77 ± 11	60 ± 24	ZATEBRADINE	0.1	88 ± 5	78 ± 6 *
	0.3	77 ± 5 *	70 ± 10		0.3	76 ± 6 *	71 ± 6 *
	1.0	65 ± 8 *	73 ± 19		1.0	63 ± 3 **	60 ± 15
PROPRANOLOL	0.03	102 ± 8	37 ± 10 *	ATENOLOL	0.01	98 ± 2	72 ± 9 *
	0.1	97 ± 8	20 ± 4 ***		0.03	90 ± 3	51 ± 13 *
	0.3	90 ± 4	14 ± 5 ***		0.1	87 ± 5	35 ± 9 **

(Means ± SEM (n=4); * P<0.05, ** P<0. 01, *** P<0.001, Student's paired t test)

The table shows HR and contractility responses to exercise, expressed as percentages of initial control responses, for cumulative doses of each drug. ZD7288, like zatebradine, reduced HR responses to exercise, with proportional decreases in contractile response, but alinidine and β-adrenoceptor antagonism depressed exercise-induced contractile responses more than expected from HR reduction alone.
The results demonstrate, as observed with zatebradine, reduction of heart rate by ZD7288 without excessive depression of contractile function. ZD7288 could prove to be a useful treatment for myocardial ischaemia.

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123P THE ROLE OF NEUTROPHILS IN REPERFUSION INJURY

J.N. Crinnion, S. Homer-Vanniasinkam, R. Hatton*, S.M. Parkin and M.J. Gough, The Department of Biomedical Science, University of Bradford and The General Infirmary at Leeds, * Cardiovascular Department, ZENECA Pharmaceuticals.

Neutrophils may be important in the pathogenesis of skeletal muscle reperfusion injury (SMRI). They may release elastase (NE) causing the subsequent disruption of the capillary basement membrane (Weiss, 1989). This study investigated the effect of both neutropaenia and the specific inhibition of neutrophil elastase upon the severity of SMRI in a rat model (Homer-Vanniasinkam et al., 1993). Gastrocnemius muscle viability (GMV- nitroblue tetrazolium), oedema (GMO- wet:dry wt) and muscle neutrophil recruitment (NR- myeloperoxidase activity- MPO) were assessed in 10 normal (no ischaemia- N), 10 ischaemic (6h unilateral hindlimb ischaemia-I) and 10 control (6h ischaemia + 4h reperfusion- C) animals. The results have been compared to 10 rats made neutropaenic with anti-neutrophil antiserum (ANS) and 8 rats receiving ELAFIN, a recombinant human endogenous elastase inhibitor (NEI, Wiedow et al., 1990) commencing 30 min prior to reperfusion (9mg/kg/4.5h, i.v.). Both groups of animals underwent the same protocol as C. ANS: neutrophils reduced from 825/mm^3 (560-1355) to 55/mm^3 (40-145), $p<0.001$. Indices of GMV and GMO were calculated (experimental/contralateral normal limb). Data is expressed as median + interquartile range.

	Viability (GMV) index	**Oedema (GMO) index**	**MPO (units/g wet wt.)**
N	1.00 (1.00-1.00)	1.00 (0.98-1.01)	0.05 (0.00-0.10)
I	1.00 (1.00-1.00)	1.01 (0.99-1.03)	0.08 (0.05-0.10)
C	0.53 (0.33-0.61)	1.61 (1.28-1.69)	2.95 (1.72-4.65)
ANS	1.00 (1.00-1.00)	1.64 (1.37-1.73)	0.30 (0.11-0.48)
NEI	1.00 (1.00-1.00)	1.52 (1.35-1.61)	0.94 (0.50-1.87)

6h ischaemia did not cause muscle infarction, oedema or NR (I v N ns) but all occurred following reperfusion (C v I, N $p<0.001$). NR (MPO) was reduced by neutrophil depletion (ANS v C $p<0.001$) and NEI ($p<0.01$ v C) with preservation of GMV (ANS, NEI $p<0.01$ v C). Reperfusion oedema still occurred. These results confirm a role for neutrophils and neutrophil elastase in the pathogenesis of post-ischaemic muscle infarction. The occurrence of significant oedema in neutropaenic animals suggests that endothelial injury may occur independently of neutrophils. Nevertheless, modification of neutrophil function may have a role in the amelioration of SMRI.

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124P EVIDENCE FOR HETEROGENEITY OF α_1-ADRENOCEPTORS IN THE RAT AORTA

P.H. van der Graaf, N.J. Welsh, N.P. Shankley & J.W. Black, Analytical Pharmacology, King's College School of Med. & Dent., Rayne Institute, 123 Coldharbour Lane, London, SE5 9NU, U.K.

At least three α_1-adrenoceptor subtypes have been identified by molecular cloning (Lomasney *et al.*, 1991). In the rat aorta, only mRNA for the α_{1A}-subtype has been detected (Lomasney *et al.*, 1991). However, there is no consistent, parallel classification from functional studies of the α_1-adrenoceptors in this tissue (Ruffolo *et al.*, 1991). In this study, the effects of a range of α_1-adrenoceptor antagonists were examined on the contractile response of the rat aorta to noradrenaline (NA). After removal of the endothelium, ring segments (4mm) of the thoracic aorta from male Wistar rats (250-300g) were mounted under 2g tension in modified Krebs buffer (37°C, 95% O_2/5% CO_2) with low [Ca^{2+}] (0.25mM) to prevent spontaneous phasic contractions. Both cocaine (30μM) and timolol (6μM) were present in all experiments. Single, NA concentration-effect (E/[A]) curves (n=5-8) were obtained by cumulative dosing in the absence or presence of antagonist (90 min. incubation).
Of the 7 antagonists, only 5-methylurapidil and YM617 (Honda *et al.*, 1985) behaved in a simple competitive manner giving parallel rightward shift of the NA E/[A] curves and associated Schild plot slopes which were not significantly different from unity. The other 5 antagonists all produced rightward shift and steepening of the NA E/[A] curves. In addition, Schild plot slope parameters estimated for HV723 (Oshita *et al.*, 1988) and phentolamine were significantly less than unity.

	$pK_B(pA_2)$	Schild plot slope (*$p<0.05$)	Effect on NA E/[A] curve slope
5-methylurapidil	7.27±0.07	0.98±0.04	-
YM617	9.67±0.10	0.98±0.06	-
prazosin	9.46±0.10	0.93±0.08	steepening
WB-4101	8.72±0.07	0.98±0.05	steepening
spiperone	8.19±0.09	0.95±0.08	steepening
HV723	(8.20)	0.84±0.05*	steepening
phentolamine	(8.00)	0.81±0.06*	steepening

Overall, these results are not consistent with simple competition at a single population of α_1-adrenoceptors in the rat aorta. The Schild regression slopes of less than unity and the steepening of E/[A] curves in the presence of antagonists are indicators of possible receptor heterogeneity and, in fact, the data could be quantitatively accounted for by a 2-receptor model (after Leff, 1987).

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125P REVERSIBLE α_1-ADRENOCEPTOR AGONIST ACTION OF CHLOROETHYLCLONIDINE IN THE RAT ANOCOCCYGEUS MUSCLE

Sithers, A.J., Marshall, R.W. and Spriggs, T.L.B., Dept Pharmacology & Therapeutics, University of Wales College of Medicine, Cardiff.

Electrically evoked contractions of the rat isolated anococcygeus muscle were attenuated in the presence of the α_1-adrenoceptor antagonists phenoxybenzamine (PBX) or prazosin but not the α_{1B}-adrenoceptor antagonist chloroethylclonidine (CEC). Indeed CEC elicited contraction of the muscle, the mechanism of which is the subject of the present investigations.

Anococcygeus muscles were dissected as described by Gillespie (1972) and mounted in Krebs solution within 7ml organ baths. Basal tension was initially set at 1g. Tissues were challenged with a 7s train of electrical pulses (10Hz, 20v, 0.8ms pulse width) at 5 minute intervals. Application of stimuli was controlled by a computer system, which synchronised tissue stimulation with a period of digitised tension data collection. Responses were also displayed on a conventional chart recorder. All experiments were performed on at least 6 individual tissues.

Contraction of the rat anococcygeus with a train of stimuli evoked a monophasic response which was completely inhibited in the presence of PBX (10nM - 10μM). Contact with 1μM prazosin similarly abolished tissue contraction. Treatment of the preparation with the α_2-antagonist yohimbine (100nM, 45mins) caused only a small increase in the response size. These results indicate that the contraction of the rat anococcygeus to trains of stimuli is mediated via postjunctional α_1-adrenoceptors.

Use of CEC at 10nM-100nM concentrations had no effect on the tissue response to stimulation. At concentrations of 1μM and 10μM, CEC rapidly produced a large and sustained contraction of the tissue. Field stimulation of the CEC-contracted preparation evoked a tissue relaxation similar to that seen in the guanethidine-treated anococcygeus muscle (Gillespie, 1972). The contractile effect of 10μM CEC was reversed with repeated washout over a 30 minute period. Yohimbine (10nM-1μM), did not reduce the CEC-induced tone of the preparation, although at a concentration of 10μM a small reduction of tissue tone was seen. Prazosin and WB4101 (10nM - 1μM) caused a marked attenuation of the CEC-induced tone, restoring the tissue to pre-CEC tension levels at concentrations of 1μM. In studies using tissues from rats pre-treated with reserpine, field stimulation produced a response 2.74±0.59% of non-reserpinised controls, whereas CEC resulted in a tissue contraction of 77.17±7.96% of control levels. These results show that CEC is acting as a reversible agonist at a postsynaptic α_1-adrenoceptor site to produce a sustained contraction of the rat anococcygeus.

Gillespie, J., (1972) *Brit. J. Pharmacol.* **45(3)**, 404.

126P TETRAETHYLAMMONIUM ENABLES ACTIVATION OF α_{1A}-ADRENOCEPTORS IN THE RAT VAS DEFERENS STIMULATED BY SINGLE ELECTRICAL FIELD PULSES

Davies, A.R., Marshall, R.W. & Spriggs, T.L.B., Dept. Pharmacology and Therapeutics, University of Wales College of Medicine, Cardiff, CF4 4XN.

We have reported (Davies *et al.*, 1992) that the use of uptake-1 inhibitors to increase synaptic overflow of noradrenaline did not induce the involvement of the putatively extra-synaptic α_{1A}-adrenoceptors in the response of the whole isolated rat vas deferens (RVD) to single pulse electrical field stimulation (SPFS). To further increase the synaptic overflow to a single pulse, in an attempt to induce a nifedipine-sensitive α_{1A}-mediated component, the release enhancer tetraethylammonium (TEA) was included in the present studies.

Stripped whole vasa from 350-470 g male Wistar rats were suspended in a Krebs-filled organ bath kept at 37°C. Following a 45 minute equilibration period and prior to data collection, the tissues were subjected to SPFS (0.8ms pulse width, 20v) until tension responses were reproducible. A computer system controlled SPFS (via parallel platinum wire electrodes) and also collected the digitised tension data. SPFS was delivered at 5 minute intervals and was followed by bath washout and drug re-administration. Results are presented as the mean + s.e. mean, (n=6) and drug effect quantified by peak tensions of the response and by the area under the curve (AuC) of the after-response between 2.0 and 9.5s following the stimulus.

TEA (2mM) produced a marked potentiation of the response of the RVD to SPFS (increase in maximum peak height from 2.16 + 0.07 to 7.38 + 0.09 g) and also produced a prolonged component, termed the "after-response" (measured by the AuC between 2 and 9.5s following the stimulus). The after-response (8.81 ± 1.03g) and potentiated peak tension were partly reduced by the P2x-purinoceptor antagonist suramin (1 mM) to 4.59 + 1.38 gs and 7.16 + 0.17 g, respectively. Prazosin (1 μM) abolished the remaining after-response (to 0.07 ± 0.14 gs), suggesting the involvement of α_1-adrenoceptors. In another experiment, the after-response produced in the combined presence of TEA (2mM), cocaine (500 nM), 17β-oestradiol (l0 μM), and suramin (1 mM) was reduced from 6.23 ± 0.85 to 2.95 ± 0.54 gs by incubation, followed by washout of the putative $\alpha_{1B/D}$-adrenoceptor alkylating agent, chloroethylclonidine (CEC, lOIIM, in the presence of 30nM yohimbine to prevent possible α_2-adrenoceptor agonism by CEC). Nifedipine (l0 μM) was then added, which not only abolished the remaining after-response (to 0.03 ± 0.01 gs), but also reduced the peak tension from 5.33 + 0.24 to 3.68 + 0. l9 g.

These results are compatible with the hypothesis that extrasynaptic α_1-adrenoceptors (putatively α_{1A}-subtype) may become involved in the response of the RVD as a result of increased release and synaptic overflow of noradrenaline.

Davies A.R., Marshall R.W., Spriggs T.L.B (1992) Br. J. Pharmacol. 107, 182P

127P TISSUE-DEPENDENT DIFFERENCES IN THE MECHANISM OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE (PDE) IV ISOENZYME INHIBITION BY BOTH ROLIPRAM AND DENBUFYLLINE

M. Shahid, K. Chipperfield & C.D. Nicholson, Dept. of Pharmacology, Organon Labs. Ltd., Lanarkshire ML1 5SH, Scotland.

PDE IV isoenzyme selective inhibitors are thought to have potential utility in the treatment of asthma (Nicholson *et al.*, 1991). The aim of the present study was to examine and compare the type of inhibition produced by the PDE IV selective inhibitors, rolipram and denbufylline (Nicholson *et al.*, 1991) on PDE IV from bovine tracheal smooth muscle (BTSM) and rat brain cortex (RBC). PDE IV isoenzymes were prepared and assayed as described previously (Shahid *et al.*, 1991). Km and Vmax vales were determined by varying [cyclic AMP] (0.1-100 μM). Ki and Ki' for the inhibitors were determined by linear plots of [inhibitor] against Km/Vmax and I/Vmax, respectively. Org 9935 (3 μM) which is a potent PDE III selective inhibitor (Shahid *et al.*, 1991) was used to inhibit minor PDE III activity in BTSM PDE IV. Both BTSM (Km: 2.97 μM) and RBC (Km: 4.25 μM) PDE IV showed Michaelis-Menten kinetics with respect to cyclic AMP with Hill plot slopes of 0.99 and 1.02, respectively. Rolipram and denbufylline produced concentration-dependent increases in Km and decreases in Vmax for BTSM PDE IV. In RBC PDE IV these compounds produced increases in Km only. Thus both rolipram and denbufylline produced mixed inhibition of PDE IV from BTSM and competitive inhibition of RBC PDE IV. The non-selective PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX) produced competitive inhibition of PDE IV from both BTSM and RBC. The kinetic constants for these inhibitors are shown in Table 1.

Table 1 Kinetic values for and mechanism of PDE IV inhibition

Tissue	Inhibitor	Mechanism	Inhibition Constants (μM) Ki	Ki'
Bovine tracheal smooth muscle	IBMX	competitive	22.0	-
	Rolipram	mixed	2.63	3.56
	Denbufylline	mixed	2.69	3.82
Rat brain cortex	IBMX	competitive	11.3	-
	Rolipram	competitive	0.99	-
	Denbufylline	competitive	1.47	-

Cloned human PDE IV contains a high-affinity rolipram binding site (Kd: 1 nM) distinct from the catalytic site (Torphy *et al.*, 1992). The presence of the former site and its influence on catalytic activity was assessed by examining the effects of low [rolipram] on inhibition of BTSM PDE IV by denbufylline. In the presence of rolipram (10 nM) denbufylline produced competitive inhibition of BTSM PDE IV (Ki: 1.70 μM). In conclusion both rolipram and denbufylline show tissue dependent differences in the mechanism of PDE IV inhibition. Furthermore the denbufylline plus low rolipram result suggests that there may be a link between the high-affinity rolipram binding and catalytic sites on BTSM PDE IV.

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Torphy, T.J. *et al.* (1992) J. Biol. Chem. **267**, 1798-1804.

128P CAN A STREPTOMYCETE ARTHROSPHORE MODEL BE OF VALUE IN DETECTING NOVEL CALCIUM-CHANNEL BLOCKING AGENTS?

G. Alderson, R.J. Hambly & T. Cross, Department of Biomedical Sciences, University of Bradford, Bradford BD7 1DP (introduced by D.Wood)

Calcium-channel blocking agents are useful clinically to control cell calcium levels in a variety of heart related and other pathological conditions. Any search for novel compounds that act as calcium antagonists would benefit from a rapid, reliable and cost effective primary screen. Present screens usually involve heart and smooth muscle contractility experiments and require animal tissues. Such methods can be costly and time consuming and, although necessary as a secondary screen, an initial inexpensive and rapid *in vitro* test could increase the number of compounds that could be assessed. Ensign *et al.* (1985), reported that two local anaesthetics, dibucaine and tetracaine, which also restrict calcium movement, inhibited the initiation of germination in *Streptomyces viridochromogenes* spores and arrested germination when administered 10 to 20 minutes after initiation. If calcium blocking agents also prevent initiation, this section of the germination process could be incorporated into a screen for the detection of novel calcium antagonists.

A semi-solidified assay medium was developed using agarose Type VII (Sigma No. A-4018), with drugs incorporated at 1mM concentrations and the germination of spores of three species of *Streptomyces* was followed by measuring changes in optical density (OD) at 580nm, which characterise the different stages of activation, initiation, swelling and emergence. In buffer alone, spores of *Stmy. viridochromogenes* did not germinate [max change in OD=3.0±0.4% (mean±s.e.mean, n=3) over 140 min]. However in defined germination medium (Hirsch & Ensign,1976) germination of spores resulted in a fall in OD which reached a maximum between 60 and 100 min (max ave fall =17.1±1.42%; e.g. drop in OD units of 54 to 45 in 70 min). Initiation of germination was prevented by both classical calcium channel antagonists, verapamil (max fall in OD=7.27±1.65%) and diltiazem (max fall in OD=5.7±0.40%), and two calcium transport inhibitors, dibucaine and tetracaine (max change in OD=3.13 ±0.46% and 2.57±0.59% respectively). No inhibitory effects were found on the initiation of germination of *Stmy. viridochromogenes* spores by lignocaine HCl (a local anaesthetic with no known calcium inhibitory effects; max fall in OD=15.8±0.21%) or atenolol (a β-blocker with no calcium inhibitory or local anaesthetic properties; max fall in OD=16.0±2.58%). Both verapamil and nifedipine inhibited the initiation process in spores of *Stmy. endus*, but nifedipine was the only antagonist found to have an inhibitory effect on the initiation process of the spores of *Stmy. albogriseolus*. This new semi-solidified medium assay system may have the potential as a rapid, fully automated high capacity screen for the detection of novel calcium antagonists and calcium transport inhibitors. In addition it could make a contribution to the investigation of the biology of streptomycete spore germination.

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Hirsch, C.F & Ensign, J.C. (1976) *J. Bacteriol.* 126, 13-23.

129P BERBERINE INHIBITS ELECTROGENIC CHLORIDE SECRETION IN RAT COLON *IN VITRO*

C. Taylor & A.W. Baird, Department of Pharmacology, University College Dublin, Dublin 4, Ireland.

Berberine is an alkaloid used in the treatment of diarrhoea. Since excessive transepithelial chloride ion secretion across intestinal epithelia is the underlying pathophysiological mechanism in secretory diarrhoea, we have examined antisecretory actions of berberine on electrogenic chloride ion secretion in isolated intestinal epithelia.

Rat colonic mucosae, stripped of underlying smooth muscle, were mounted in Ussing chambers (window area = 0.63cm^2) and voltage clamped by continuous application of short circuit current (SCC). Epithelial ion transport was stimulated by addition of secretagogues to the basolateral solutions. Paired preparations were used, n=5 throughout. Statistical analysis was carried out using Student's t-tests or analysis of variance; *p<0.05, ** p<0.01, *** p<0.005.

Carbachol (1-300μM) produced a concentration dependent increase in SCC (EC50 = 5.9±0.9μM)which can be accounted for by electrogenic secretion of chloride ions. Berberine (100μM and 500μM) significantly inhibited responses to carbachol in a non-competitive manner (p<0.01 in each case). Anti-rat IgE antibodies which activate lamina propria mast cells stimulated an inward SCC of 17.5±5.5 μA which was significantly reduced in the presence of 100μM and 500μM berberine by 90.4±3.2% (p<0.05) and 93.9±5.3% (p<0.01) respectively. We further examined the action of berberine on chloride secretion stimulated by activation of intracellular regulatory pathways. Cyclic AMP-dependent chloride secretion was stimulated using the stable analogue dibutyryl cyclic AMP and forskolin which activates adenylate cyclase. Calcium-dependent chloride secretion was stimulated using thapsigargin (Brayden *et al.*,1989). Results (expressed in μA) are shown in Table 1. Comparisons have been made between berberine treated and control tissues .

Table 1.

Stimulus	Control	Berberine (100μM)	Berberine (500μM)
Forskolin (10μM)	39.8 ± 5.4	27.3 ± 8.0 *	8.71 ± 4.8 **
db cyclic AMP (500μM)	63.0 ± 17.3	25.9 ± 10.5 n.s.d.	18.8 ± 5.5 *
Thapsigargin (3μM)	27.6 ± 6.6	7.3 ± 5.0 n.s.d.	-6.7 ± 0.9 ***

In summary, berberine also reduced SCC responses to direct activation of chloride secretion by cyclic AMP (forskolin) and calcium (thapsigargin). The alkaloid appears to be acting at a distal (epithelial) site in regulating chloride channel opening. It is not yet clear whether cyclic AMP and calcium pathways are independently influenced or whether some interaction between them (Cliff and Frizzell, 1990) accounts for the apparently non-selective inhibition of chloride secretion by berberine.

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130P COMPARATIVE EFFECTS OF POTASSIUM CHANNEL OPENERS ON ^{86}RUBIDIUM EFFLUX FROM RAT ILEUM

M.P. Davies, J.R. McCurrie & D. Wood, Postgraduate Studies in Pharmacology, School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP

In previous studies examining the actions of potassium channel openers (KCOs) on longitudinal muscle-myenteric plexus preparations (LMMP) of rat ileum, we found that BRL 38227 (BRL) and pinacidil (PIN) relaxed contractions induced by KCl (15-20mM) and by electrical field stimulation: minoxidil sulphate ($MxSO_4$) acted similarly but was of very low potency and diazoxide (DZ) was without effect in this system (Davies *et al.*, 1991). The present study investigates the effects of these KCOs on ^{86}Rubidium efflux (RE) from LMMP, as a measure of K channel opening (Bolton & Clapp, 1984).

LMMP was prepared from distal ileum of male rats (Paton & Vizi, 1969). 1cm sections were incubated for 3h at 37°C in Krebs' solution containing ^{86}Rb (1.67μCi ml^{-1}) and gassed with 95% O_2/5% CO_2. During the subsequent 60 min efflux period (from t_o min) the sections were transferred at 4 min intervals through a series of tubes containing Krebs' solution with or without KCOs or appropriate vehicles. After 60 min, the ^{86}Rb remaining in the sections and in all efflux media was assessed using a gamma scintillation spectrometer. RE was calculated as the fractional loss of ^{86}Rb from each tissue, expressed as % min^{-1}, of the total amount of ^{86}Rb present in that tissue during each 4 min of the efflux period.

In control experiments, RE declined gradually over time. BRL 30μM, a concentration which in preliminary studies under identical conditions caused a substantial and significant increase in RE from rat portal vein preparations, was without effect on RE from LMMP, compared with vehicle- or time-controls (n = 6). Addition of KCl (20mM) to the efflux medium (from t_{+16} min) produced an immediate doubling in RE from LMMP which was sustained over the course of the experiment: e.g. at t_{+28} min, RE rates were 2.1 ± 0.2% min^{-1} in control (n = 6), 4.2 ± 0.3% min^{-1} in KCl-stimulated tissues (n = 5) (means ± s.e. means, P<0.01). In the presence of KCl , BRL (30μM, included from t_{+28} min) now caused an immediate, although transient, increase in RE rates, to 4.6 ± 0.4% min^{-1} at t_{+32} min (n = 6), compared with a fall in control tissues exposed only to KCl, to 3.9 ± 0.3%min^{-1} at t_{+32} min (n = 5, P<0.05). This protocol was subsequently adopted for study of all KCOs.

None of the KCOs significantly increased RE at concentrations below 30μM. BRL and PIN, 30-300μM, gave concentration-related increases in RE. At 100μM, BRL and PIN increased RE to 5.2 ± 0.4 and 4.7 ± 0.2% min^{-1} respectively at t_{+32} min (P<0.01 compared with control). $MxSO_4$ increased RE (to 4.5 ± 0.2% min^{-1}, P<0.05) at 300μM only, whilst DZ (30-500μM) was ineffective (n ≥4 at each concentration of KCO).

These results support our findings in functional studies using the KCOs (Davies *et al.*, 1991), although the concentrations required to elicit increases in ^{86}Rb efflux are considerably greater than those required to cause maximal inhibition of functional responses. ^{86}Rb efflux mechanisms operating in intestine appear to differ from those in vascular smooth muscle, as indicated by the requirement for inclusion of KCl in the efflux medium for LMMP.

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131P THE EFFECT OF A SELECTIVE THROMBIN INHIBITOR ON LIPOPOLYSACCHARIDE-INDUCED DISSEMINATED INTRAVASCULAR COAGULATION IN THE ANAESTHETISED RAT

A.J. Ford and D.J Longridge. Department of Pharmacology, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS U.K.

Surgical operation, trauma, systemic bacterial infections, and other states of stress predispose to thrombotic complications. The blood of such patients becomes hypercoagulable and therefore shows an increased tendency to form thrombi. Subsequent intravascular clotting may be localised or manifest as disseminated intravascular coagulation (DIC). Antithrombotic agents such as heparin are widely used for the treatment of DIC but are limited by: the requirement of co-factors for activity; inactivity against thrombin bound to fibrin; lack of oral efficacy (Knabb et al., 1992). Since the intravascular generation of thrombin is considered to be the essential pathogenic factor in DIC, this enzyme is a logical therapeutic target, with specific thrombin inhibitors potentially unhindered by the limitations of heparin therapy. One such agent, D-MePhe-Pro-Arg-H (GYKI-14766) is a synthetic, tripeptide, reversible thrombin inhibitor (Bagdy et al., 1992), with reported oral activity (Green et al., 1992).

We evaluated the efficacy of GYKI-14766 compared to standard unfractionated heparin in a model of lipopolysaccharide (LPS)-induced DIC in the anaesthetised male Wistar rat with respect to ^{125}I-fibrin deposition and blood cell changes. Doses of heparin and GYKI-14766 were chosen for their equivalent efficacy at prolonging thrombin-time: GYKI-14766: 0.25 mg/kg i.v. bolus + 0.25 mg/kg/h i.v. infusion (5 h); Heparin: 50 U/kg i.v. bolus + 50 U/kg/h i.v. infusion (5 h). LPS (*E.coli*) was administered at 10 mg/kg/h i.v. infusion (1 h), starting concomitantly with heparin/GYKI administration. Treatment groups were compared to saline/LPS and saline/saline controls. Whole blood samples were periodically analysed for changes in white blood cell and platelet counts. Animals were killed four hours after the end of the LPS infusion. Selected organs were analysed for ^{125}I-fibrin content and deposition ratios (DR) were calculated compared to final blood ^{125}I levels.

Parameter	Time point	Saline/Saline (Grp 1)	Saline/LPS (Grp 2)	Heparin/LPS (Grp 3)	GYKI-14766/LPS (Grp 4)
Spleen DR	4 h post LPS	0.20 ± 0.03	0.82 ± 0.29 (a)	0.25 ± 0.01	0.76 ± 0.19 (a,c)
Kidney DR	4 h post LPS	0.12 ± 0.01	0.22 ± 0.05 (a)	0.15 ± 0.01	0.23 ± 0.04 (a,c)
Heart DR	4 h post LPS	0.16 ± 0.01	0.19 ± 0.01 (a)	0.17 ± 0.02 (b)	0.19 ± 0.01
Liver DR	4 h post LPS	0.20 ± 0.04	0.65 ± .015 (a)	0.31 ± 0.06 (b)	0.65 ± 0.12 (a,c)
% Change WBC	End LPS (1h)	-12 ± 11	-53 ± 6 (a)	-45 ± 6 (a)	-49 ± 5 (a)
(From time 0)	4 h post LPS	-1 ± 8	-42 ± 8 (a)	-28 ± 16	+9 ± 17 (d)
% Change Platelet Count	End LPS (1h)	-17 ± 8	-20 ± 4	-17 ± 7	-20 ± 4
(From time 0)	4 h post LPS	-12 ± 5	-44 ± 4 (a)	-36 ± 8 (a)	-43 ± 5 (a)

Data are mean ± sem, n=6 per group. (a) = $p<0.05$ vs Grp 1; (b) = $p<0.10$ vs Grp 2; (d) = $p<0.05$ vs Grp 2, (c) = $p<0.05$ vs Grp 3; Mann-Whitney U-test).

At equivalent anti-thrombin doses, heparin compared to GYKI-14766 alleviated the fibrin deposition stimulated by LPS infusion. Whilst the initial leukopenia seen by the end of the LPS infusion was not affected by either treatment, the total white cell count in the GYKI group showed significantly more recovery by the end of the experiment. Neither treatment had any effect upon the observed thrombocytopenia. These results indicate that the coagulation responses seen in this model are not solely thrombin-dependent. The ability of heparin to inhibit other factors in the coagulation cascade (e.g. Factor Xa) may explain the alleviation of the fibrin deposition by this agent. Since the evolution of DIC may involve a complex multi-mediator system, specific single agents (e.g. anti-thrombins) alone may not be capable of alleviating this syndrome.

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132P CHARACTERISATION OF THE 5-HT RECEPTOR MEDIATING ELECTROGENIC FLUID SECRETION IN HUMAN SMALL INTESTINE

D.E. Burleigh & R.A. Borman, Department of Pharmacology, Queen Mary & Westfield College, Mile End Road, London E1 4NS

5-hydroxytryptamine (5-HT) has previously been shown to produce fluid secretion at all levels of the intestine. It's effects are attributed to both enhanced electrogenic chloride secretion and diminished electroneutral sodium absorption. *In vitro*, serosal application of 5-HT to isolated sheets of intestinal mucosa causes an increase in short-circuit current (SCC) (Brown & Miller, 1991). The present investigation attempts to determine the mechanism of action of 5-HT on electrogenic ion transport in human ileal mucosa.

Mucosal sheets of terminal ileum were mounted in Ussing chambers and clamped at zero potential by a high impedance voltage clamp. Transmucosal SCC was continuously monitored and used as a measure of electrogenic fluid secretion. Two cumulative 5-HT concentration-response curves (0.26-100 μM) were obtained, the second curve being carried out either in the presence of antagonist or control vehicle. In control experiments, application of 5-HT gave a maximal rise in SCC of 65.4 ± 3.0 μAcm^{-2}. The influence of tetrodotoxin and various 5-HT antagonists on basal SCC and the SCC response to 5-HT is shown below.

Treatment	(μM)	Basal SCC[a] (μAcm^{-2})	5-HT Concentration Ratio[b]	n
Control		+9.4 ± 7.5	3.93 (2.38-6.49)	16
Tetrodotoxin	(3.1)	-8.6 ± 3.0*	6.40 (2.72-15.05)	6
Methysergide	(10)	+26.3 ± 1.7*	4.71 (1.70-13.09)	6
+Ketanserin	(1)			
Ondansetron	(10)	+10.6 ± 9.2	2.35 (0.51-10.78)	6
SDZ 205-557	(1)	-1.9 ± 5.2	18.90 (7.85-45.55)*	6

[a] Basal SCC expressed as arithmetic mean ± s.e.mean. [b] 5-HT concentration ratio between two successive response curves, given as geometric mean with 95% confidence limits. Data tested for significance using Mann-Whitney U Test; * indicates $p < 0.05$.

Only SDZ 205-557 (a 5-HT_4 antagonist) significantly increased the concentration ratio between two successive response curves. In conclusion, it appears that electrogenic fluid secretion in human isolated ileal mucosa is mediated by a 5-HT_4 receptor.

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133P EFFECT OF 5-HT_3/5-HT_4 RECEPTOR LIGANDS ON *IN VITRO* K+-STIMULATED [^{3}H]5-HT RELEASE FROM RAT CORTICAL SLICES

C.H.K. Cheng, B. Costall, K. Mason, R.J. Naylor and J.A. Rudd Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, BD7 1DP, U.K.

Microdialysis studies have demonstrated a differential activity of R(+)- and S(-)-zacopride to modify the extracellular levels of 5-hydroxytryptamine (5-HT) from the rat frontal cortex (Barnes *et al.*, 1992). The present study investigates whether R(+)- and S(-)-zacopride have a similar stereoselective action to modify 'in vitro' K^+ stimulated [^{3}H]5-HT release from rat cortical slices. The effect of 5-methoxytryptamine, renzapride, tropisetron and ondansetron was also investigated.

The cerebral cortex obtained from female Hooded-Lister rats (200-250g, Bradford bred) were dissected out and cross-chopped (0.35 x 0.35 mm x thickness of cortical ribbon). The slices was pre-depolarized with 25 mM K^+ Krebs buffer for 20 min and then incubated with 1.0 nM [^{3}H]5-HT (28.6 Ci $mmol^{-1}$) in the presence of 10 µM pargyline for 20 min at 37°C. Tissue slices were transferred into superfusion tissue chambers and perfused with Krebs buffer containing 1.0 µM fluoxetine. After a 30 min washout period, 4 min samples of perfusate were collected over 72 min. At t = 12 min (S1) and 48 min (S2), the slices were stimulated with 25 mM K^+ Krebs buffer for 4 min. Drugs were added 4 min prior to and throughout the S2 stimulation. The tritium content remaining in the tissue slices and of each fraction was determined by liquid scintillation spectroscopy. Results (S2/S1 ratio) were calculated as described by Barnes *et al.* (1989).

Table 1. Effect of 5-HT_3/5-HT_4 receptor ligands on 'in vitro' K^+ stimulated [^{3}H]5-HT release (S2/S1 ratio) from rat cortical slices

Treatment	Control	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5} (M)
5-methoxytryptamine	0.76±0.02	0.75±0.02	0.66±0.03*	0.54±0.01*	0.46±0.05*	0.49±0.02*
R(+)-zacopride	0.70±0.02	0.70±0.09	0.68±0.02	0.68±0.15	0.74±0.07	0.85±0.07
S(-)-zacopride	0.75±0.02	0.77±0.04	0.72±0.02	0.74±0.04	0.77±0.03	0.72±0.04
Renzapride	0.81±0.08	0.82±0.05	0.85±0.07	0.77±0.06	0.97±0.18	0.75±0.05
Tropisetron	0.77±0.02	0.78±0.06	0.69±0.03	0.73±0.05	0.83±0.03	0.81±0.02
Ondansetron	0.89±0.06	0.88±0.02	0.90±0.04	0.84±0.04	0.95±0.04	0.86±0.06

Data represent the mean ± s.e.mean of three to four separate experiments in which each treatment was carried out in triplicate. *P<0.05-0.01, significant differences compared to the respective control group (ANOVA, followed by Dunnett's t test).

5-Methoxytryptamine caused a concentration-dependent reduction in K^+ stimulated [^{3}H]5-HT release from rat cortical slices (Table 1). The inability of the 5-HT_3/5-HT_4 receptor agonists/antagonists to modify K^+ stimulated [^{3}H]5-HT release is consistent with their failure to modify extracellular levels of 5-HT during dialysis studies (Barnes *et al.*, 1992, Ge *et al.*, 1992), with the exception of R(+)-zacopride which decreases extracellular levels of 5-HT 'in vivo'.

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134P (R) AND (S) 56532: MIXED 5-HT_3 AND 5-HT_4 RECEPTOR LIGANDS WITH OPPOSING ENANTIOMERIC SELECTIVITY

E. Leung, R. Alvarez, D.W. Bonhaus, R.D. Clark[1], L.G. Johnson, C-H. Lee, W.L. Smith, E.H.F. Wong, R.L. Whiting and R.M. Eglen. Institute of Pharmacology, and [1]Institute of Organic Chemistry, Syntex Discovery Research, 3401 Hillview Avenue, Palo Alto, CA 94304, USA

Some 5-HT_3 antagonists including benzimidazolones such as BIMU-1 and BIMU-8, or benzamides, including metoclopramide and zacopride, also act as agonists at the 5-HT_4 receptor. Zacopride exhibits similar enantiomeric activity at these receptors, i.e. (**S**) > (**R**). We have studied the activity of (**S**) and (**R**) enantiomers of RS 56532 {6-amino-5-chloro-2-(1-azabicyclo[2,2,2]octan-3-yl)2,3-dihydro-1H-benz [de] isoquinoline-1,3-dione hydrochloride}, a hybrid structure of zacopride and the novel 5-HT_3 receptor antagonist, RS 42358 (Wong et al., 1993).

At 5-HT_3 receptors mediating contractions of guinea-pig isolated ileum, both enantiomers lacked intrinsic activity (1 nM - 10 µM). (**R**) and (S) RS 56532 exhibited pK_B (± s.e. mean) values against 5-HT of 7.9 ± 0.1 and < 5.0, respectively. In radioligand binding studies using [^{3}H]quipazine and rat cerebral cortical membranes, (**R**) and (**S**) RS 56532 exhibited pK_I values of 9.1 ± 0.1 (n=3) and 8.0 ± 0.1 (n=3), respectively. In the anaesthetized rat, (**R**) and (S) RS 56532 inhibited the von Bezold Jarisch reflex with ID_{50} (95 % confidence limits) values of 5 (3 - 7) and 78 (47 - 128) µg kg^{-1}, i.v., respectively.

At 5-HT_4 receptors mediating relaxations of isolated rat oesophagus, the (**S**) enantiomer was more potent (pEC_{50}=7.9 (7.7 - 8.4)) than the (**R**) enantiomer (pEC_{50} < 6.0). Responses to (**S**) RS 56532 were antagonized by GR 113808 (Grossman et al., 1993), the slope of the Schild regression was not significantly different from unity and a constrained pA_2 value of 9.1 (9.0 - 9.3) was obtained. (**S**) and (**R**) RS 56532 displaced [^{3}H]GR 113808 binding in guinea-pig striatal membranes with pK_I values of 7.6 ± 0.03 (n=3) and 6.5 ± 0.05 (n=3), respectively. At 5-HT_4 receptors mediating enhanced adenylyl cyclase activity in guinea-pig hippocampus, a similar enantiomeric selectivity was observed, i.e. (**S**) > (**R**) (pEC_{50} values were 6.3 and 4.5, respectively). In the anaesthetized, bilaterally vagotomised micropig, (**S**) RS 56532 elicited a tachycardia response (ED_{50} (95% confidence limits = 16 (11 - 21) µg kg^{-1}, i.v.). An increase in heart rate (28 beats min^{-1}) was observed with (**R**) RS 56532 only at 1 mg kg^{-1}, i.v.

In summary, RS 56532 exhibited opposing enantiomeric selectivity at 5-HT_3 (**R** > **S**) and 5-HT_4 receptors (**S** > **R**) both *in vitro* and *in vivo*. These enantiomers may be useful for predicting differences in the binding domains of the two receptors.

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135P 5-HT RECEPTORS AND 5-HYDROXYTRYPTOPHAN-EVOKED DEFAECATION IN MICE

Banner, S.E., Smith, M.I. & Sanger, G.J., SmithKline Beecham Pharmaceuticals, Harlow, Essex, CM19 5AD, UK.

5-HT has been implicated in disturbed colonic motility. For example, in volunteers 5-hydroxytryptophan (5-HTP) mimicked the increased bowel activity seen in irritable bowel syndrome (IBS) patients (Davidson et al, 1957). Here we attempt to characterise the 5-HT receptors involved in the 5-HTP induced increase in faecal pellet output in mice.

Fed male CD1 mice (28-34g) were housed individually in mesh-bottomed perspex boxes mounted 5cm above the bench for 20min. Subsequently, methiothepin, ketanserin, mianserin (30-100μgkg^{-1} sc), granisetron (100-1000μgkg^{-1} sc) or vehicle (saline) were dosed 5min prior to saline or 5-HTP (10$mgkg^{-1}$) sc. Faecal pellet output (FPO) numbers were recorded at 10min intervals and cumulative values calculated for each mouse against time-matched controls.

In vehicle treated mice 5-HTP significantly increased FPO, rising to a maximum at 20min post-dose (336 ± 13% increase, $p<0.001$, $n=130$) compared with saline. At this time point methiothepin, ketanserin, mianserin (each at 30μgkg^{-1}) and granisetron (100μgkg^{-1}) had no significant effect on FPO in either saline or 5-HTP treated mice. However, methiothepin (100μgkg^{-1}) inhibited FPO in saline (80 ± 15% inhibition, $p=0.01$, $n=10$) and 5-HTP treated mice (65 ± 20% inhibition, $p=0.007$, $n=5$). Similarly, ketanserin (100μgkg^{-1}) reduced FPO in saline (60 ± 22%, $p=0.06$, $n=10$) and 5-HTP treated mice (25 ± 17%, $p=0.18$, $n=10$). Mianserin (100μgkg^{-1}) tended to reduce FPO in saline treated mice (48 ± 31%, $p=0.16$, $n=9$ of 10) and 5-HTP treated mice (33 ± 12%, $p=0.03$, $n=15$). Granisetron (1000μgkg^{-1}) reduced FPO in saline (53 ± 24%, $p=0.1$, $n=10$) but not in 5-HTP treated mice (38 ± 27%, $p=0.28$, $n=5$).

More than one 5-HT receptor may mediate 5-HTP evoked defaecation in mice. The 5-HT_1 and 5-HT_2 receptor antagonists have little or no consistent effect at low doses, but caused non-selective inhibition at high, non-specific doses (Connor et al, 1986). 5-HT_3 receptors may be involved in normal defaecation, but a high dose granisetron did not antagonise the 5-HTP evoked increase in FPO. These results suggest that 5-HT_1, 5-HT_2 and 5-HT_3 receptors do not play a major role in the mechanisms by which 5-HTP increases the rate of defaecation in mice, therefore these receptors are unlikely to be involved in the pathophysiology of diarrhoea predominant IBS. Consequently, an investigation into the role of the 5-HT_4 receptor is warranted (Banner et al, 1993).

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136P ASSESSMENT OF 5-HT_3 RECEPTOR RECOGNITION SITE LEVELS IN THE PUTAMEN OF PATIENTS WITH HUNTINGTON'S CHOREA AND PARKINSON'S DISEASE

K. E. Bufton, L. J. Steward, N. M. Barnes, Department of Pharmacology, The Medical School, University of Birmingham, Birmingham B15 2TT UK.

Whilst clinical development of selective 5-HT_3 receptor (5-hydroxytryptamine$_3$ receptor) antagonists progresses, the available evidence indicates that the distribution of 5-HT_3 receptor recognition sites (5-HT_3-Rs) in human forebrain is unlike that of any other species reported to date (e.g. Barnes et al., 1989; Abi-Dargham et al., 1993), with relatively high levels of 5-HT_3-Rs within the basal ganglia (Abi-Dargham et al., 1993). The present studies investigated the levels of 5-HT_3-Rs in the putamen from patients with Parkinson's disease (PD) and Huntington's chorea (HC) to determine whether 5-HT_3-Rs are primarily located on dopamine neurones and/or on neurones that have their cell bodies within this area.

Putamen tissue from patients with clinically diagnosed HC and PD and patients who had died without a neurological or psychiatric disorder (control) were prepared for binding assays essentially as described previously (Barnes et al., 1989).

For [^{3}H]granisetron binding, assay tubes contained competing drug or vehicle and [^{3}H]granisetron. Brain homogenate was added to initiate binding which proceeded at 37°C for 30 min before termination by filtration and washing. Radioactivity on the filters was assayed by liquid scintillation spectroscopy.

[^{3}H]Granisetron (0.9-1.0 nM) specific binding (defined by ondansetron, 10.0 µM) was detected in putamen homogenates from control patients and those with HC and PD (Table 1).

Group	[^{3}H]Granisetron binding (fmol/g)
Control (HC)	72±6
Huntington's chorea	39±8*
Control (PD)	68±4
Parkinson's disease	65±8

Table 1. Specific [^{3}H]granisetron binding in putamen homogenates from patients with HC or PD and their respective control groups. Data represents the mean±SEM fmol/g wet weight tissue, n=8-10. *P=0.004, Students t test. In the HC group, two values were omitted to generate the mean value (115 and 126 fmol/g).

The failure of the neuronal degeneration associated with PD to reduce 5-HT_3-R levels in the putamen indicates that these receptors are not primarily located on dopamine terminals within this brain region. The reduction in 5-HT_3-R levels in putamen homogenates from patients with HC, however, indicates that at least a proportion of the 5-HT_3-R population in the human putamen is located on neurones that have their cell bodies within this area.

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137P FURTHER CHARACTERISATION OF AN ATYPICAL 5-HT RECEPTOR MEDIATING ENDOTHELIUM-DEPENDENT VASORELAXATION

G. R. Martin, C. Browning & H. Giles, Biochemical Sciences, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS.

Two distinct 5-HT receptor types mediate endothelium-dependent responses to 5-HT. One (pig coronary artery: Schoeffter & Hoyer, 1990) resembles the 5-HT_{1D} sub-type, but the other (rabbit jugular vein: Leff *et al*, 1987) is an 'orphan' of the present nomenclature and remains to be classified. Here we further define the pharmacology of the atypical 5-HT receptor in rabbit jugular vein and compare its profile with the 5-HT_{2C} (formerly 5-HT_{1C}) receptor in pig choroid plexus (Hoyer *et al*, 1989; Schoeffter & Hoyer, 1989, see Table 1). Endothelium-dependent relaxations were measured as isometric force changes in rings of rabbit external jugular vein prepared as detailed elsewhere (Leff *et al*, 1987). Agonist cumulative concentration-effect curves were constructed in tissues tonically contracted with U-46619 (10nM); antagonist effects were determined following 60 min. incubation with tissues.

Table 1. Agonist and antagonist potencies at the endothelial 5-HT receptor in rabbit jugular vein and 5-HT_{2C} receptor in pig choroid plexus (n≥3).

Agonists	Rabbit Jugular Vein p[A_{50}]	max.	Pig Choroid Plexus p[A_{50}]	max.	5-HT_{2C} pKi
5-HT	8.55	1.00	6.47	1.00	7.50
(±)α-Me-5-HT	8.34	1.04	7.31	1.14	7.20
5-CT	7.16	0.90	5.65	1.01	6.20
sumatriptan	<4.50	-	4.25	0.06	4.10
m-CPP	6.80	0.46	6.87	0.65	7.70
TFMPP	6.88	0.46	6.78	0.79	7.20
RU24969	6.64	0.84	6.22	0.36	6.50
SCH23390	7.01	0.88	5.70	0.37	8.30
723C86[$]	7.47	1.01	-	-	6.50
(+)DOI	7.66	1.09	7.02	0.58	7.70

Antagonists	Rabbit Jugular Vein pK_B	slope	Pig Choroid Plexus pK_B	5-HT_{2C} (pKi)
Methysergide	NSA ≤ 10.0	-	8.85	8.6
Methiothepin	NSA ≤ 9.50	-	8.18	7.6
Cyproheptadine	NSA ≤ 7.50	-	7.86	7.9
Mesulergine	NSA ≤ 7.30	-	8.88	8.8
Metergoline	NSA ≤ 8.50	-	10.59	9.2
Yohimbine	6.21± 0.12	1.07	<5.00	4.4
Spiperone	NSA ≤ 6.00	-	4.90	5.90
Ketanserin	<5.00	-	6.57	7.00
Tropisetron	6.45± 0.05	0.96	-	4.60
DAU 6285	<5.00	-	-	-

$: (±)1-[5-(2-thenyloxy)-1H-indol-3-yl]propan-2-amine; NSA = non-surmountable antagonism at the -log[antagonist] shown.

These results show that whilst the atypical endothelial 5-HT receptor shares pharmacological similarities with the 5-HT_{2C} receptor, differences, especially in antagonist potencies, imply that the receptors are not the same. Whether or not these discrepancies reflect authentic receptor subtypes, or the existence of species homologues of the 5-HT_{2C} receptor awaits comparative information on the molecular and biochemical properties of these receptors.

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138P ENDOGENOUS AND EXOGENOUS 5-HT ACCESS DIFFERENT RECEPTORS TO DEPRESS THE MONOSYNAPTIC REFLEX

Crick, H and Wallis, D.I. Dept. Physiol., UWCC, Cardiff, CF1 1SS.

5-HT-induced depressions of a spinal monosynaptic reflex (MSR) may be mediated by more than one 5-HT receptor subtype . The 5-HT uptake inhibitors, citalopram and fluvoxamine, were employed in studies of the isolated, hemisected spinal cord of the 5 day old rat. The MSR (amplitude 1-5mV) was recorded in the ventral root via suction electrodes following supramaximal stimulation of the dorsal root (0.067Hz). 0.1μM citalopram caused a large shift to the left of the 5-HT dose-response curve (Crick and Wallis, 1991). 1μM citalopram, like fluvoxamine (1μM), caused a depression of the MSR, which was reversed by the 5-HT_{2A}/5-HT_{2C} antagonists ketanserin and ritanserin (both at 1μM) (Crick and Wallis, 1991). p-Chloroamphetamine (p-CA) (1μM) mimicked the action of the uptake inhibitors in causing complete supression of the MSR and this was partially reversed by ketanserin (1μM). Citalopram-induced depression of the MSR was not significantly altered by strychnine (1μM), suggesting the absence of inhibition mediated by glycinergic interneurones. The action of these agents may thus lead to the accumulation of endogenous 5-HT which appears to depress the MSR via a 5-HT_{2A}/5-HT_{2C} receptor. Indeed, the agonist alpha-methyl 5-HT caused a dose-dependent inhibition of the MSR (IC_{50} = 8.9 ± 2.2μM, n=4). However, 5-HT dose-response curves could be obtained both in the presence and absence of citalopram 1μM/ketanserin 1μM and fluvoxamine 1μM/ketanserin 1μM (Table. 1).

Table. 1. IC_{50} values for 5-HT before and after blockade of 5-HT uptake and 5-HT_{2A}/5-HT_{2C} receptors.

	Control	Cital 1μM/Ket 1μM	n	Control	Fluvox 1μM/Ket 1μM	n
MSR	9.5 ± 3.2μM	96 ± 18.0nM*	3	9.67 ± 1.3μM	79.7 ± 1.8nM**	4

* $p < 0.05$ ** $p < 0.005$

We have reported elsewhere that MSR depression by exogenous 5-HT is not antagonised by ritanserin, methiothepin and spiperone (all at 1μM) (Crick and Wallis, 1993) suggesting that exogenous 5-HT may act at a site other than 5-HT_1 subtypes or 5-HT_2 receptors. On the assumption that a mixed population of receptors may be responsible, blockade of 5-HT_{2A}/5-HT_{2C} receptors might allow pharmacological investigation of the further site(s). However, IC_{50} values for 5-HT depression in the presence of citalopram 0.1μM/ritanserin 1μM (88 ± 39nM) and on addition of the selective 5-HT_{1A} antagonist spiroxatrine (1μM) (88 ± 24nM, n=3) were not significantly different. Also, 5-HT-induced depression in the presence of citalopram 0.1μM/ketanserin 1μM (IC_{50} = 160 ± 90nM) was not significantly altered upon addition of the non-selective ligand quipazine, 1μM (147 ± 90nM, n=3). The action of exogenous 5-HT is mimicked by 8-OH DPAT, TFMPP, 5-CT (Crick and Wallis, 1991) and sumatriptan (unpublished data). Thus, the results presented here suggest that whilst endogenous 5-HT may act through 5-HT_{2A}/5-HT_{2C} receptors to cause MSR depression, exogenous 5-HT is not blocked by antagonists at this site nor by spiroxatrine, quipazine, methiothepin and spiperone and may act at a second receptor still to be fully characterized.

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T.J.O. Sayer, K.F. Martin[1] & P.H. Redfern, Pharmacology Group, School of Pharmacy and Pharmacology, University of Bath and [1]Boots Pharmaceuticals Research Department, Nottingham.

The central serotoninergic system, in the rat, possesses both somatodendritic (5-HT_{1A}) and terminal ($5HT_{1B}$) autoreceptors, which modify the terminal release of 5-hydroxytryptamine (5-HT). Tryptophan hydroxylase, the activity of which shows circadian variation, is the rate limiting step in the synthesis of 5-HT, and both types of autoreceptors have been shown to regulate its activity (Hamon *et al.*, 1973; Fernstrom *et al.*, 1990). It is therefore possible that the circadian variation in the activity of this enzyme is influenced by autoreceptor activity. We have investigated the effect of the $5\text{-HT}_{1A/1B}$ agonist, 5-methoxy-3(1,2,3,6-tetrahydro-4-pyridyl)-1H-indole (RU24969), on the accumulation of 5-hydroxytryptophan (5-HTP) after decarboxylase inhibition, as a measure of tryptophan hydroxylase activity at four equally spaced time points in the light:dark cycle.

Male Wistar rats (240-260g) received either (±)cyanopindolol (CP) (3mg/kg i.p.) then 30 min later RU24969 (i.p.) or CP (i.p.) followed by saline after 30 mins or RU24969 (i.p.) alone. All animals received m-hydroxybenzylhydrazine (NSD 1015) (100mg/kg i.p.) 30 min after the RU24969 or saline, and were killed after another 30 min. The concentration of 5-HTP in hypothalamic homogenates was assayed by HPLC coupled to ECD; protein concentrations were determined from the pellet. Results are presented in table 1.

	Mid Dark	End Dark	Mid Light	End Light
Basal levels	1.10±0.08	0.76±0.10	1.13±0.10	0.91±0.09
RU24969 (3mg/kg)	1.14±0.05	0.73±0.06	1.08±0.05	0.87±0.02
RU24969 (9mg/kg)	0.84±0.06*	0.52±0.07*	0.80±0.01**	0.66±0.03*
CP + RU24969 (9mg/kg)	1.21±0.05	0.66±0.06	1.50±0.03**a,b	0.90±0.01
CP + SALINE	1.07±0.03	0.80±0.02	1.13±0.1	1.00±0.03

Table 1. Levels of 5-HTP are expressed in ng 5-HTP/μg protein as mean ±s.e.mean, n≥3. Significance determined using two way ANOVA followed by Studentised Range test; (*) $p<0.05$ (**) $p<0.01$ within groups, [a] $p<0.05$ Mid Light compared to End Light or Mid Dark and [b] $p<0.01$ Mid Light compared to End Dark.

Basal levels of 5-HTP did not differ significantly over 24 hours. RU24969 significantly reduced 5-HTP levels at 9mg/kg at each time point, and this effect was abolished when rats were pretreated with CP. These results show that the 5-HT_{1B} autoreceptor can influence 5-HT synthesis, but that the degree of inhibition does not show a circadian variation. CP alone has no effect on 5-HTP accumulation indicating that there is no autoinhibitory tone in the rat hypothalamus.

TJOS is a SERC CASE student in conjunction with Boots Pharmaceuticals.

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140P COMPARISON OF ADENOSINE POTENTIATION BY DIPYRIDAMOLE IN THE ATRIOVENTRICULAR AND SINOATRIAL NODES AND ATRIAL MUSCLE OF THE GUINEA-PIG

B.J. Meester, D.J. Prentice, N.J. Welsh, N.P. Shankley, J.W. Black, Analytical Pharmacology, The Rayne Institute, King's College School of Medicine and Dentistry, 123 Coldharbour Lane, London SE5 9NU

Adenosine (ADO) exerts negative dromotropic, chronotropic, and inotropic effects in the atrioventricular (AV) and sinoatrial (SA) nodes and left atrium by acting on ADO A_1-receptors (Belardinelli *et al.*, 1982; Collis, 1983). Extracellular ADO is removed by a rapid carrier-mediated uptake system (Nees *et al.*, 1985), which can be blocked by dipyridamole (DIP). The ADO-enhancing actions of DIP in the AV and SA nodes and left atrium of the guinea-pig were studied, using concentration-effect (E/[A]) curves to the non-selective ADO receptor agonist 5'-(N-ethyl)carboxamidoadenosine (NECA), which is not a substrate for uptake, for comparison.

Isolated hearts from male Dunkin-Hartley guinea-pigs (200-300g) were perfused with Krebs-Henseleit buffer and gassed with 95% O_2/5%CO_2 at 35°C. Isolated right atria and left atria (guinea-pigs 300-400g) were suspended in 20ml organ baths at 37°C and 31°C, respectively. Single agonist E/[A] curves were obtained in the absence or presence of DIP (incubation: AV node 30 min, SA node and left atrium 60 min).

Table 1. Comparison of ADO uptake in the AV and SA nodes and left atrium of the guinea-pig

Tissue	[DIP]	ADO log [A_{50}]	ADO + DIP log [A_{50}]	NECA log [A_{50}]	ADO + DIP / NECA log [A_{50}] ratio
AV node	1μM	-5.12 ± 0.15	-5.90 ± 0.09	-6.76 ± 0.12	0.86 ± 0.15
SA node	3μM	-3.62 ± 0.10	-6.27 ± 0.05	-7.34 ± 0.05	1.07 ± 0.07
LA	3μM	-4.60 ± 0.27	-6.10 ± 0.09	-7.22 ± 0.03	1.12 ± 0.09

Mean ± s.e.mean (n=4-6)

The agonist potency ratio between adenosine in the presence of dipyridamole and NECA was not significantly different between tissues confirming that adenosine uptake was saturated. In the presence of dipyridamole, adenosine had a similar potency across assays. The low potency of adenosine in the absence of dipyridamole suggests that adenosine uptake has a much larger capacity in the sinoatrial node compared to atrioventricular node and left atrial muscle.

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141P COMPARISON OF ADENOSINE RECEPTORS MEDIATING NEGATIVE INOTROPY IN GUINEA-PIG AND HUMAN ATRIAL PREPARATIONS

V.J. Barrett, D.J. Prentice, J.F. Martin[1] & G.R .Martin, Biochemical Sciences, Wellcome Research Labs., Beckenham, Kent BR3 3BS. and [1]Department of Medicine, King's College School of Medicine & Dentistry , Denmark Hill, London SE5 8RX.

The existence of adenosine receptors in the human heart has been established (Böhm *et al.*, 1985). In atrial tissue stimulated by isoprenaline both adenosine and R-PIA (R-(-)-N^6-phenylisopropyladenosine) produce negative inotropic responses which are antagonised by the non-selective P_1 receptor antagonist, 8-PT (8-phenyltheophylline) yielding a pA_2 value consistent with adenosine receptor blockade (Böhm *et al.*,1989). Negative inotropic responses to adenosine in the guinea-pig are generally accepted as being A_1 receptor mediated (Collis, 1983). In this study we have compared receptors mediating negative inotropy in the guinea-pig and human atrium using a series of adenosine agonists and DPCPX (1,3-dipropyl,8-cyclopentylxanthine), an A_1 selective antagonist (Martinson *et al.*,1987).

Human right atrial appendages obtained from patients undergoing cardiac bypass surgery or aortic valve replacement were set up according to Gille *et al.*, (1985). 10nM isoprenaline was added to the baths to increase force of contraction before construction of agonist concentration-effect (E/[A]) curves. For the antagonist study E/[A] curves were repeated after exposure to DPCPX for 60 minutes. Guinea-pig left atria were set up according to Collis (1983), except that tissues were maintained at 31°C and stimulation was delivered (1Hz, 1ms duration) at supramaximal voltage. One curve per preparation was constructed in the presence of dipyridamole (3μ M). For the antagonist studies curves were constructed after a 60 minute incubation with DPCPX. Agonist potency estimates obtained in both tissues were as follows:

	$p[A_{50}]$ (-log[M], n=4/5)	
AGONIST	HUMAN	GUINEA-PIG
R-PIA	6.91±0.04	6.93±0.08
NECA	7.15±0.12	7.11±0.08
CPA	7.38±0.12	7.39±0.10
2-CADO	6.84±0.09	6.40±0.07

Data are expressed as mean ± s.e.mean.
NECA=5'-(N-ethyl)carboxamidoadenosine; CPA=N^6-cyclopentyladenosine; 2-CADO=2-chloroadenosine.

Agonist potency orders obtained in both tissues were not different. The similar potency in each case of NECA, PIA and CPA is indicative of an action at A_1 receptors; at A_2 receptors NECA is 20-50 times more potent than the N^6 substituted compounds (Collis, 1983). Moreover DPCPX behaved as a competitive antagonist in atria from human and guinea-pig and did not differentiate between receptors in both tissues (Human pK_B=8.24±0.11, Schild slope=1.14±0.27, d.f.=10, Guinea-pig pK_B=8.33±0.11, Schild slope=0.96±0.13, d.f.=16.).

These results indicate that the adenosine receptors in human and guinea-pig atria are both of the A_1 subtype.

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142P ROLE OF Ca^{2+} IN THE NEGATIVE INOTROPIC RESPONSE OF GUINEA-PIG LEFT ATRIA TO A_1-RECEPTOR AGONISTS

WR Ford & KJ Broadley. Department of Pharmacology, Welsh School of Pharmacy, UWCC, PO Box 13, Cardiff, CF1 3XF

The negative inotropic response to adenosine via A_1-receptors in guinea-pig left atria is thought to involve two ionic currents mediated through different mechanisms. Activation of the acetylcholine-regulated potassium current ($I_{k\text{-}ACh}$) causes a shortening of the cardiac action potential duration allowing less time for calcium entry into the cells, and direct inhibition of calcium current (I_{Ca}) reduces calcium entry into the atrial cells, resulting in negative inotropy (Belardinelli and Isenberg 1983). $I_{k\text{-}ACh}$ is thought to be the more important (Cerbai *et al* 1988). The aim of the current study was to examine the role of I_{Ca} in the functional response of guinea-pig left atria to A_1-receptor stimulation.

'Resting state' and partially depolarised guinea-pig isolated paced left atria were used. 'Resting state' atria were paced at 2Hz using a voltage 50% above threshold with a pulse width of 1ms.Partially depolarised atria were paced at 0.2Hz using 100 volts with a pulse width of 10ms. Partial depolarisation was effected by raising the concentration of KCl to 27mM, ionically compensating by reducing NaCl to 96.1mM in the Krebs bicarbonate buffer. Cumulative dose-response curves were constructed with N^6-(2-phenylisopropyl)adenosine ((-)PIA). Time course studies were conducted using a single bolus dose of 10^{-6}M (-)PIA. Blockers, where used, were incubated for at least 20 minutes before agonist addition.

The IC40 value for the negative inotropic action of (-)PIA in 'resting state' left atria was 1.2(0.4-3.5) x 10^{-7}M (mean±95% confidence interval) with 78.8±5.7% (n=5) (mean±s.e.m) maximal inhibition of initial tension . These were significantly less than the IC40 of 3.3(1.0-10.4) x 10^{-6}M and a 55.2±3.7% (n=6) maximal inhibition of initial tension in partially depolarised tissue ($P<0.05$, Student's t-test). This suggests the absence of a component of the response in depolarised tissue. The residual response is assumed to be solely due to inhibition of Ca^{2+} influx. Support for a minor K^+-mediated component of the response of normal atria to (-)PIA was indicated by the effect of the K^+ channel blocker, 4-aminopyridine (4AP, 10mM). This slowed the rate of onset of the response to a single dose of (-)PIA (10^{-6}M), the inhibition of tension between 0.4 and 3 minutes being significantly less ($P<0.05$). In partially depolarised atria, however, 4AP had no effect upon the time course. Similarly, 4AP did not significantly affect the IC40 (7.3(0.6-95.4)x10^{-7}M, n=4) or the maximal response (55.6±5.0%) of (-)PIA on partially depolarised atria ($P<0.05$).

The possibility that a high stimulation voltage would lead to catecholamine release was investigated by constructing cumulative dose-response curves to (-)PIA in the presence of 10^{-6}M atenolol. The IC40 for (-)PIA in the presence of atenolol was 7.0(1.7-28.9) x 10^{-6}M with a maximal inhibition of 54.3±5.1% neither of which was significantly different from (-)PIA alone, indicating the absence of an antiadrenergic effect of (-)PIA.

In partially depolarised atria, the contractions are mediated by slow inward Ca^{2+} current (Thyrum, 1974). Thus the Ca^{2+} channel antagonist, amlodipine was effective in causing a negative inotropic response in both normal (IC40, 1.8 (0.5-2.3) x 10^{-6}M, max inhibition 96.2±1.1%, n=6) and partially depolarised atria (IC40, 1.5 (0.77-3.03) x 10^{-7}M, max inhibition 55.6±5.0%, n=4). The similarity in profile of activity between (-)PIA and amlodipine suggests that a major component of the response to (-)PIA is due to Ca^{2+} channel blockade.

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143P COMPARISON OF A_1 AND A_2 ADENOSINE RECEPTOR AGONISTS IN THE TREATMENT OF ISCHAEMIA-INDUCED LEFT VENTRICULAR DYSFUNCTION

A.S. Clanachan, B.A. Finegan[1] & F. Moustarah, Departments of Pharmacology and Anaesthesia[1], University of Alberta, Edmonton, Alberta, Canada, T6G2H7

Acute left ventricular dysfunction (LVD) may be managed by vasodilators that reduce impedance to LV ejection (afterload) and thereby increase cardiac index (CI). Our previous studies have shown that adenosine 5′-monophosphate (AMP), a soluble adenosine (Ado) prodrug, has advantages over sodium nitroprusside (SNP) because, at equieffective afterload reducing doses, CI was increased more by AMP than by SNP (Finegan & Clanachan, 1991). Ado stimulates two main types of receptors (A_1, present in the myocardium and A_2, present in the vasculature). While both A_1 and A_2 Ado receptor agonists cause systemic vasodilatation and afterload reduction (Gerencer *et al.*, 1992), A_1 Ado receptor agonists have the ability to protect the heart from the consequences of ischaemia. Therefore, to determine the contribution of these potential mechanisms to the beneficial effect of Ado, we have compared the effects of selective A_1 and A_2 Ado receptor agonists in a canine model of LVD.

LVD was induced in closed chest, anaesthetised dogs by the injection of glass microbeads into the left main coronary artery. LVD was associated with reductions in CI (by 35%, $P < 0.0001$) and systemic vascular conductance index (SVCI, by 29%, $P < 0.0001$). After the induction of LVD, graded doses of N^6-cyclopentyladenosine (CPA, 680-fold A_1-selective, 20 to 1000 $\mu g\ kg^{-1}$, n = 7) and CGS-21680 (170-fold A_2-selective, 1 to 100 $\mu g\ kg^{-1}$, n = 7) caused significant, dose-dependent decreases in mean arterial pressure (up to 35 ± 7% and 27 ± 3%, respectively, $P < 0.0001$) and increases in SVCI (up to 105 ± 32% and 158 ± 22%, respectively, $P < 0.0001$). CGS-21680 caused a greater increase in CI (up to 86 ± 15%, $P < 0.05$) than that produced by CPA (up to 24 ± 19%, $P < 0.05$). Cardiodepressant properties (bradycardia) of CPA were evident. In contrast to Ado and CPA, CGS-21680 reduced preload by up to 45 ± 10%, as judged by LV end diastolic pressure. When compared at doses causing equieffective increases in SVCI, CGS-21680 elicited an increase in CI that was similar to that for AMP whereas CPA was significantly less effective despite its potential to exert a direct anti-ischaemic action. Interestingly, the ability of CGS-21680 to elevate CI despite reducing preload suggests that the previously observed difference between AMP- and SNP-induced enhancement of CI was not solely due to their contrasting effects on venous tone.

Thus, in a canine model of acute LVD, these A_1- or A_2-selective Ado receptor agonists did not provide any additional benefit over AMP alone. The desirable haemodynamic profile of AMP suggests that AMP (or Ado) may be useful in the therapeutic management of acute LVD.

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144P ADENOSINE A_1 RECEPTOR-MEDIATED CONTRACTIONS IN RAT COLON ARE BOTH INHIBITED AND POTENTIATED BY 8-PHENYLTHEOPHYLLINE (8-PT)

JJ Reeves, J Coates, JE Jarvis and MJ Sheehan, Dept of Gastrointestinal Pharmacology, Glaxo Group Research Ltd., Ware, Herts, SG12 0DP.

The purine agonist 5′-N-ethylcarboxamidoadenosine (NECA) mediates contraction in rat isolated colon via adenosine A_1 receptors (Bailey et al., 1992). In the present study we have investigated further the contraction to a range of adenosine receptor agonists and its modification by adenosine receptor antagonists.

Experiments were carried out on rat isolated colonic muscularis mucosae (rat colon) as described by Bailey et al., (1992). Sequential additions of NECA (0.01-10μM) produced concentration-dependent contractions (maximum 0.44±0.06g at 3μM) with a mean EC_{50} value of 0.16 (0.14-0.19)μM (n=17), in rat colon. These responses were mimicked by the A_1-selective agonists N-(1S, trans)-[2-hydroxycyclopentyl] adenosine (GR79236, n=9), and N^6(R-phenylisopropyl) adenosine (R-PIA, n=11) and by the A_2-selective agonists N-[(2-methylphenyl)methyl] adenosine (metrifudil, n=3) and 2[p-(2-carboxylethyl) phenethyl amino]-5′N-ethyl carboxamidoadenosine (CGS21680, n=4) (Gurden et al., 1993). R-PIA produced only 76.7±3.0% of the maximum NECA contraction. The rank order of agonist activity (equieffective concentration-ratio where NECA=1) was R-PIA (0.49) = GR79236 (0.57) ≥ NECA (1.0) >> metrifudil (54) > CGS21680 (165). The contractions elicited by each agonist were inhibited by the A_1-selective antagonist 8-cyclopentyl 1,3 dipropylxanthine (DPCPX, n=4) with apparent pK_B values between 9.2 and 9.5. Reductions in the NECA maximum were observed with DPCPX at 3-10nM. The non-selective adenosine antagonist 8-PT (0.1-3μM) not only shifted the NECA curves to the right [pA_2 = 6.7 (6.4-7.3), slope = 0.7 (0.4-1.1), n=20] but also increased the NECA maximum (by up to 93.0±10.2% with 3μM 8-PT). 8-PT (1μM) also produced increases in the maximum response obtained to GR79236 and R-PIA. 8-PT (3μM) neither had any direct effect nor affected contractions to carbachol in rat colon. In the presence of 8-PT (1μM), DPCPX (3-10nM) produced further rightward shifts of the contractions to NECA with a mean apparent pK_B of 9.3 (8.7-9.8), (n=5). Neither NECA (10μM), metrifudil (100μM) nor CGS21680 (100μM) produced any relaxation of the rat colon pre-contracted with carbachol (1μM) in the absence or presence of DPCPX (0.1μM, n=3).

The results confirm the presence of an adenosine A_1 receptor mediating contraction in rat colon. In addition, the present study has shown that 8-PT (but not DPCPX) augments this contraction. One possible explanation of this enhancement is that 8-PT may be preferentially antagonising an adenosine receptor which mediates relaxation in rat colon thereby increasing the contractile component to A_1 agonism. However, we have been unable to reveal any relaxation in this preparation.

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145P HYPOTENSIVE RESPONSES TO THE PUTATIVE ADENOSINE A_3 RECEPTOR AGONIST, N^6-2-(4-AMINOPHENYL)ETHYL ADENOSINE (APNEA) IN THE RAT

A.M. Carruthers & J.R. Fozard, Preclinical Research, Sandoz Pharma Ltd., CH-4002 Basel, Switzerland

Recently, Zhou et al. (1992) described the cloning, expression and pharmacological characterisation of a novel adenosine receptor which they designated A_3. The site could be labelled with high affinity by [^{125}I]-N^6-2-(4-aminophenyl)ethyl adenosine ([^{125}I]-APNEA). The non-iodinated derivative, APNEA, has since been shown to induce hypotension in the angiotensin II-supported circulation of the pithed rat; xanthine resistance and the agonist pharmacology of this response suggest mediation by the A_3 receptor (Fozard and Carruthers, 1993a). Here, we analyse further the cardiovascular effects of APNEA in the rat.

Male Sprague-Dawley rats (279-401 g) were anaesthetized with pentobarbitone sodium, 60 mgkg^{-1} i.p., pithed (where specified) and prepared for recording blood pressure (BP) and heart rate (HR) as described in detail previously (Fozard & Carruthers, 1993b). In pithed animals, BP was maintained at 100-120 mmHg by i.v. administration of either angiotensin II (0.2-0.5 μgkg^{-1}min^{-1}, phenylephrine (4-5 μgkg^{-1}min^{-1} after propranolol 1 mgkg^{-1}), clonidine (10-20 μgkg^{-1}min^{-1} after prazosin 1 mgkg^{-1}) or N^G-nitro-L-arginine methyl ester (L-NAME; 10 mgkg^{-1} as a bolus injection). APNEA, 1-30 μgkg^{-1} was injected cumulatively i.v.

APNEA induced broadly similar dose-related hypotensive effects in anaesthetized (ED_{30} 7.8 ± 0.6 μg/kg, n=7) rats and in pithed preparations infused or injected with angiotensin II (ED_{30} 6.4 ± 0.4 μg/kg, n=4) phenylephrine (ED_{30} 9.7 ± 1.2 μgkg^{-1}, n=3), clonidine (ED_{30} 15.5 ± 5.5 μgkg^{-1}, n=4) or L-NAME (6.4 ± 0.4 μgkg^{-1}, n=3). APNEA also induced weak and transient (< 2 min) bradycardia (range of group means, 16-28 % at 30 μg/kg). Pretreatment (5 min) with 8-(p-sulphophenyl)theophylline, 40 mgkg^{-1} i.v., abolished the bradycardia but had minimal effects on the hypotension in any group. Pretreatment with the angiotensin converting enzyme inhibitor, spirapril, 5 mgkg^{-1} i.p. plus 1 mgkg^{-1} i.v., or glibenclamide, 20 mgkg^{-1} i.v. prior to injecting APNEA in angiotensin II-supported pithed rats failed to influence the falls in HR and BP induced by APNEA.

Thus, the 8-SPT-resistant hypotensive response to APNEA is manifested in anaesthetized animals and occurs independently of the pressor agent used to restore blood pressure to normal in pithed preparations. The data also suggest that neither suppression of the renin-angiotensin system nor activation of glibenclamide-sensitive ATP-dependent potassium channels can explain the putative A_3 receptor-mediated hypotensive response to APNEA.

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146P CHARACTERISATION OF THE RESPONSES OF EQUINE DIGITAL VEINS TO ADENOSINE AND ITS ANALOGUES

J.Elliott & J. Soydan, Department of Veterinary Basic Sciences, Royal Veterinary College, Royal College Street, London NW1 0TU.

Equine laminitis (EL) is a common vascular problem in veterinary medicine. It is now accepted that decreased capillary blood flow to the sensitive laminae underlies EL. Under such ischaemic conditions, production of adenosine and its release from the oxygen starved tissues will increase. The vascular effects of adenosine have not been studied in the horse. The aim of the present study was to examine the effects of adenosine in equine digital veins (EDV) and to characterise the receptors involved in producing these actions.

Rings of EDV were denuded of their endothelium and prepared for isometric tension recording as previously described (Bryant *et al.* 1992). 8-Phenyltheophylline (8-PT; 1 μM) was added 30 min prior to the addition of agonists. Tone was induced by the addition of U44069 (9, 11-dideoxy-9α, 11α-epoxymethano-prostaglandin $F_{2\alpha}$; 30 nM). Cumulative dose response curves (CRCs) were constructed to adenosine (ADE; 0.01 μM to 0.1 mM), 5'-N-ethylcarboxamidoadenosine (NECA; 0.1 nM to 1 μM), 5'-N-methylcarboxamidoadenosine (MECA; 1 nM to 10 μM), N^6-cyclopentyladenosine (CPA; 0.01 μM to 0.1 mM), adenosine triphosphate (ATP; 0.01 μM to 1 mM) and 2-methylthio-ATP (Me-ATP; 0.01 to 10 μM).

The isometric tension induced by U44069 was 3.55 ± 0.17 g and did not differ significantly in the presence or absence of 8-PT. All the agonists examined, except Me-ATP, produced dose-dependent relaxations reducing U44069-induced tone by more than 90% of its original value. Me-ATP produced a maximum reduction in U44069 tone of 8.7%. The order of potency and estimated EC_{50} values for the remaining agonists were NECA (9.1 ± 2.1 nM; n=5), MECA (62.1 ± 22.6 nM; n=8), CPA (1.64 ± 0.2 μM; n=8), ADE (2.8 ± 0.6 μM; n=24) and ATP (10 ± 2.2 μM; n=24). At concentrations of 10 μM and above, ATP produced transient increases in tension followed by sustained relaxation. 8-PT (1 μM) produced a parallel shift to the right in the CRCs for all agonists tested with no change in the maximum response producing dose ratios of 17.7 ± 2.9 (MECA), 13.0 ± 3.2 (CPA), 4.1 ± 1.0 (ADE) and 4.8 ± 1.4 (ATP). When compared with its effect on ADE, 8-PT produced significantly greater increases in the EC_{50} values for MECA and CPA.

EDVs possess adenosine receptors, activation of which leads to vasodilation. The order of potency of agonists examined in the present study suggests these receptors are of the A_2 subtype (Olsson & Pearson, 1990). The vasorelaxant action of ATP seems likely to be mediated by its metabolism to adenosine. An additional vasorelaxant action of adenosine at an intracellular site could explain the less potent inhibitory effect of 8-PT versus ADE when compared with its analogues.

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147P INHIBITORY EFFECT OF OCTREOTIDE AND SEGLITIDE ON VASODEPRESSOR RESPONSES INDUCED BY COLONIC DISTENSION IN ANAESTHETISED RATS

E. McKeen, W. Feniuk, P. P. A. Humphrey, J. Gale[1] and A. Green[1].
Glaxo Institute of Applied Pharmacology, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ and [1]Department of Gastrointestinal Pharmacology, Glaxo Group Research, Ware, SG12 0DJ.

Distension of the gastrointestinal tract by elevating intraluminal pressure produces vasodepressor responses thought to be mediated by activation of pain reflexes (Lembeck and Skofitsch, 1982). Somatostatin (SS) is found in the gastrointestinal tract and may be involved in sensory neurotransmission (Stine et al., 1982). We have investigated the effect of the stable SS analogues octreotide and seglitide on vascular responses to colonic distension in anaesthetised rats.

Male rats AHA Wistar (approx. 300g) were anaesthetised with pentobarbitone (60mg kg^{-1} ip) supplemented by continuous infusion (6mg/h) into a tail vein. Arterial pressure was recorded from a carotid artery. A saline filled balloon was inserted (1.5-2cm) into the sigmoid colon and drugs administered into a jugular vein.

Colonic distension (0.5-2ml) produced a volume dependent vasodepressor response (max. decrease in diastolic blood pressure by a 2ml volume was 41 ± 6 mm Hg; mean ± se mean, n=6) which were reproducible and usually accompanied by a bradycardia. The vasodepressor response produced by a 2ml distension (37 ± 2 mm Hg) was converted to a small pressor response of 12 ± 3 mm Hg (n=4) by morphine (5mg kg^{-1} iv). This effect of morphine was reversed by naloxone (5mg kg^{-1} iv, n=4).

Octreotide (10-100μg kg^{-1} iv) produced a transient (<3min) decrease in arterial blood pressure (36 ± 4 mm Hg at 100μg kg^{-1} iv). 5 min later, vasodepressor responses to colonic distension (1.5ml) were markedly attenuated (controls 40 ± 2 mm Hg; Oct 100μg kg^{-1} iv 8 ± 5 mm Hg, n=4). Responses to distension recovered by approximately 75% over the next hour. Subsequent administration of octreotide (100μg kg^{-1} iv) had no effect on responses to colonic distension. In naloxone (5 mg kg^{-1} iv) pretreated animals, the inhibitory actions of octreotide remained unchanged.

In three of six experiments, seglitide (100μg kg^{-1} iv) produced a similar effect to octreotide on vasodepressor responses to a 1.5ml colonic distension (control vasodepressor response 32 ± 7 mm Hg and 7 ± 3 mm Hg after seglitide). In the other three experiments, seglitide had no effect but prevented the inhibitory action of octreotide (100μg kg^{-1} iv); subsequent administration of morphine (5mg kg^{-1} iv) reduced vasodepressor responses to colonic distension (32 ± 8 mm Hg before morphine; 3 ± 2 mm Hg after morphine).

The mechanism underlying the inhibitory effect of octreotide on responses to colonic distension is unknown but may correlate with its reported analgesic action (Penn et al., 1992). The inhibitory effects of octreotide and seglitide appear to be mediated via a common mechanism which is subject to tachyphylaxis and distinct from that of morphine.

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148P IS THE RENAL PAPILLA INVOLVED IN THE ANTIHYPERTENSIVE EFFECT OF THE ANGIOTENSIN AT_1 RECEPTOR ANTAGONIST, GR117289?

A. Travers, M.J. Robertson, K.L. Clark & G.M. Drew. Department of Cardiovascular and Respiratory Pharmacology, Glaxo Group Research, Ware, Herts, UK.

A hormonal antihypertensive system of renal origin has been postulated, a key component of which is thought to be medullipin, released from renomedullary interstitial cells (RIC) of the renal papilla. Transplanted RIC reduce blood pressure in hypertensive rats, and angiotensin II has been reported to inhibit this action (Muirhead, 1991). It is therefore possible that the antihypertensive activity of angiotensin receptor antagonists is partly due to disinhibition of the effects of angiotensin II on the medullipin system. 2-bromoethylammonium bromide (BEA) selectively destroys the renal papilla, and thus the medullipin system (see Muirhead, 1991). We have therefore examined the effect of BEA pre-treatment on the antihypertensive action of the angiotensin AT_1 receptor antagonist, GR117289 (Hilditch et al., 1991), in a high-renin model of hypertension; 6 day renal artery ligated hypertensive (RALH) rat (Travers & Hilditch, 1992). Rats were pre-treated with either BEA (200mg/kg i.p.) or its vehicle, saline (10ml/kg i.p.) five weeks prior to the experiment. Diastolic blood pressure (DBP) was measured via a carotid artery catheter in conscious animals. Only rats in which DBP was 140mmHg or more were used, and DBP measurements were made 1, 3, 5, 7 and 24h after administration of GR117289 (3mg/kg i.a.).

In vehicle pre-treated RALH rats, GR117289 produced a marked reduction in DBP that persisted for more than 24h (Figure 1). BEA pre-treatment induced selective papillary necrosis in approximately 40% of RALH rats, with little visual effect on the cortex. The extent of papillary necrosis and the lack of cortical damage was determined histologically at the end of the experiment. In those rats with total papillary necrosis, the antihypertensive effect of GR117289 was not significantly reduced ($P>0.05$, Figure 1). This provides circumstantial evidence against the involvement of medullipin in the antihypertensive effect of AT_1 receptor antagonists such as GR117289 in RALH rats.

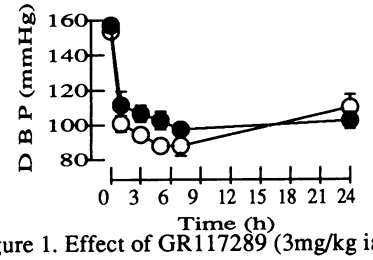

Figure 1. Effect of GR117289 (3mg/kg ia) on DBP in saline (○;n=9) or BEA (●;n=10) pre-treated RALH rats. Values are mean ± s.e. mean.

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149P INHIBITION OF DIATRIZOATE-INDUCED REDUCTION IN RENAL BLOOD FLOW BY AN ENDOTHELIN ANTAGONIST

S-J Slee & C. Wilson; Zeneca Pharmaceuticals, Cardiovascular Department, Alderley Park, Macclesfield, Cheshire, SK10 4TG.

The clinical use of radio-contrast media (RCM) can be associated with the initiation of acute renal failure, possibly due to effects on renal haemodynamics (Porter, 1990). Since elevated circulating levels of the vasoconstrictor endothelin (ET) have been reported following administration of RCM in rats (Heyman et al., 1992), we have examined the effects of the ET antagonist BQ123 (Ihara et al., 1992) on diatrizoate (a high osmolar RCM) induced cardiovascular effects.

Alderley Park rats (320-380g) were anaesthetised with Intraval (100 mg kg^{-1} ip) and the right jugular vein and carotid artery were cannulated for administration of drugs and measurement of mean arterial pressure (MAP) respectively. Blood flow was assessed using a pulsed Doppler flow probe positioned around the left renal artery. All animals received indomethacin (5 mg kg^{-1}) 5 min prior to RCM. In some animals, BQ123 (1 mg kg^{-1}) was dosed 2 min prior to administration of diatrizoate (Urografin 325; 8.9 ml kg^{-1} infused over 2.5 min resulting in a final dose of 2.9g iodine kg^{-1}). Changes in MAP and renal conductance (CR) were calculated.

			% pretreatment control				
		Pretreatment	Time after starting administration of diatrizoate (min)				
		Control	2.5	5	10	20	30
MAP (mmHg)	Control (n=6)	129 ± 6	67 ± 7 *	89 ± 2 *	94 ± 3	96 ± 2 *	94 ± 1 *
	BQ123 (n=6)	123 ± 2	70 ± 3 *	84 ± 5 *	85 ± 6 *	83 ± 4 *	89 ± 6 *
CR ([kHz $mmHg^{-1}$]10^3)	Control (n=6)	22.7 ± 3.1	121 ± 7 *	85 ± 6 *	70 ± 6 *	80 ± 5 *	86 ± 5 *
	BQ123 (n=6)	30.3 ± 6.9	140 ± 12	101 ± 6	95 ± 4 †	99 ± 5 †	98 ± 5

* $p < 0.05$, paired Students t-test vs pretreatment control; † $p < 0.05$ unpaired Students t-test vs control group.

During the period of infusion of RCM, MAP decreased and remained below pretreatment values throughout the period studied. This effect of RCM was not antagonised by BQ123. The effects of RCM on CR were biphasic. The initial increase in conductance seen during the period of infusion was not antagonized by BQ123. The peak reduction in CR was to 70% of control 10 min after commencing administration. This was significantly attenuated by BQ123.

The ability of the ET receptor antagonist BQ123 to attenuate diatrizoate induced decreases in CR provides evidence for a role of endothelin in this response.

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150P THE ENDOTHELIN RECEPTOR ANTAGONISTS BQ-123 AND PD 145065 REVERSE ESTABLISHED VASOCONSTRICTOR RESPONSES TO ENDOTHELIN-1 *IN VITRO*

Graham H. Allcock, Timothy D. Warner and John R. Vane. *The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ, U.K.*

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). Thus, it has appeared probable that selective antagonists may act poorly against established responses to ET-1. Here, we have assessed *in vitro* 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., 1992) in reversing vasoconstrictions induced by ET-1 in the rat kidney or thoracic aorta.

Male Wistar rats (200-350 g) were anaesthetised with pentobarbitone sodium (60 mg/kg, i.p.). For the isolated perfused kidney (IPK), the right renal artery was cannulated, the kidney removed, perfused (10 ml min^{-1}) with Krebs' buffer (plus 0.1 % BSA) and perfusion pressure recorded. Rings of thoracic aorta (RTA) were also prepared and suspended in organ baths (resting tension, 1g) for isometric measurement of contractions (Warner et al., 1992). After an equilibration period (IPK, 30 min; RTA, 60 min) approx. 80% of maximum constrictor responses were induced in the vasculature of the IPK with ET-1 ($3x10^{-10}$ M) or methoxamine ($2x10^{-5}$ M), and in the RTA with ET-1 ($3x10^{-9}$ M) or phenylephrine ($3x10^{-7}$ M). Cumulative concentrations of BQ-123 or PD 145065 (10^{-9} to 10^{-6} M) or vehicle were then infused into the IPK. Similarly, BQ-123 or PD 145065 (10^{-5} M), or vehicle was added to the Krebs' buffer bathing the RTA.

Either BQ-123 or PD 145065 reversed the pressor effects of ET-1 in the IPK (thresholds approx. 10^{-8} M) such that at 10^{-6} M they reduced the elevated perfusion pressure by 56.9±8.8 % and 22.8±8%, respectively (n=4-5). Vehicle was without effect, and neither BQ-123 nor PD 145065 reduced constrictions of the IPK induced by methoxamine (n=4). Similarly, both antagonists reversed constrictions of the RTA induced by ET-1. For instance, at 20 and 40 min after addition of antagonist the tone was respectively reduced by 52.8±12.3% and 85.8±5.6% with PD 145065, and by 43.8±12.2% and 77.1±6.7% with BQ-123. Vehicle was without effect, and constrictions of the RTA induced by phenylephrine were unaffected by either BQ-123 or PD 145065.

Thus, both BQ-123 and PD 145065 reverse established responses to ET-1 mediated by either mixed ET_A and ET_B receptors (IPK; Wellings et al, 1993) or by ET_A receptors (RTA; Warner et al., 1992), suggesting that they can displace ET-1 from either receptor.

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151P HUMAN α AND β CGRP-MEDIATED RESPONSES *IN VITRO*: SENSITIVITY TO THE CGRP RECEPTOR ANTAGONIST, $CGRP_{8-37}$

A. Butler, D.T. Beattie & H.E. Connor, Department of Neuropharmacology, Glaxo Group Research Ltd., Park Road, Ware, Herts SG12 0DP.

Human α and β calcitonin gene-related peptide (h.α and βCGRP) have been suggested, on the basis of differential sensitivity to the CGRP receptor antagonist, $CGRP_{8-37}$, to act via different receptors; h.αCGRP-mediated dilatation of guinea-pig and human cerebral blood vessels is inhibited by $CGRP_{8-37}$, while that evoked by h.βCGRP is not (Jansen, 1992; Jansen et al., 1992). This proposal was investigated further using tissues which exhibit responses to both h.α and βCGRP.

Isometric changes in tension were recorded conventionally from rabbit hepatic and dog basilar artery rings precontracted submaximally (to approximately 75% of the maximum) with phenylephrine (10μM) and prostaglandin $F_{2\alpha}$ (3μM) respectively. Electrically-evoked (20V, 2msec and 0.1Hz) twitches of the rat vas deferens were recorded isometrically. Cumulative concentration-response curves to h.α and βCGRP (0.01nM-0.1μM) were constructed in each tissue in the absence and presence of $CGRP_{8-37}$ (1μM). Apparent pKb values were determined to assess antagonist potency.

h.α and βCGRP produced concentration-dependent relaxations of the rabbit hepatic and dog basilar arteries, and inhibited electrically-evoked twitches in the rat vas deferens. The compounds produced the same maximum response and were similar in potency in the dog basilar artery (pEC_{25} values for h.α and βCGRP, 8.4±0.2 (n=7) and 8.2±0.2 (n=10) respectively). In the rabbit hepatic artery h.βCGRP was significantly ($p<0.05$, student's t-test) more potent than h.αCGRP (pEC_{50} values for h.α and βCGRP, 8.9±0.1 (n=5) and 9.3±0.1 (n=5) respectively) whilst in the rat vas deferens h.αCGRP was significantly ($p<0.05$) more potent than h.βCGRP (pEC_{50} values, 8.0±0.1 (n=6) and 7.3±0.1 (n=6) respectively). $CGRP_{8-37}$ (1μM) antagonised responses to both h.α and βCGRP in the rabbit hepatic (apparent pKb values, 7.5±0.2 (n=5) and 7.2±0.3 (n=5) respectively) and dog basilar arteries (apparent pKb values, 6.7±0.2 (n=5) and 6.4±0.2 (n=6) respectively), the antagonist potency against both h.α and βCGRP being significantly greater ($p<0.05$) in the rabbit hepatic artery compared to the dog basilar artery. The h.α and βCGRP-induced inhibition of twitches in the rat vas deferens were unaffected by $CGRP_{8-37}$ (1μM, dose-ratios of 1.4±0.1 (n=6) and 0.6±0.1 (n=5) respectively).

The present investigation, therefore, has found no evidence to suggest that the receptors mediating responses to h.α and βCGRP in the rabbit hepatic and dog basilar arteries and rat vas deferens can be differentiated in terms of their sensitivity to the CGRP receptor antagonist, $CGRP_{8-37}$. In the rat vas deferens, $CGRP_{8-37}$ had no effect on responses to either h.α or β CGRP indicating that the receptors in this tissue are different from those in the rabbit hepatic and dog basilar arteries.

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152P THE P_1-PURINOCEPTOR OF THE GUINEA-PIG TAENIA COLI

A. S. Kelley & M. Hollingsworth, Smooth Muscle Pharmacology Group, School of Biological Sciences, Manchester University, Manchester, M13 9PT.

The guinea pig taenia coli contains P_1- and P_{2Y} -purinoceptors, both mediating relaxation (Burnstock & Kennedy, 1985). Our initial studies have been concerned with P_1-purinoceptors.

Taenia coli, 15-20 mm long, were mounted for isometric recording in organ baths containing Krebs' solution and 10 μM indomethacin maintained at 37°C and gassed with 95% O_2, 5% CO_2. A control log concentration effect curve was constructed to each agonist, usually against a carbachol (50 nM) spasm, and repeated after 30 min equilibration with a modifying drug.

When adenosine was added cumulatively in the presence of carbachol or KCl (25 mM) an extremely low potency was recorded (carbachol : $_pD_2 = 3.30 \pm 0.07$, $E_{max} = 72.5 \pm 8.2\%$, n=7; KCl : $_pD_2 < 3$, $E_{max} = 37.7 \pm 6.9\%$, n = 8). In the presence of the adenosine uptake inhibitor nitrobenzylthioinosine (NBTI, 300 nM) (Clanachan et al, 1987) adenosine, tested against a KCl spasm, was potentiated but the E_{max} was unchanged. Non-cumulative addition of adenosine in the presence of carbachol yielded a $_pD_2$ of 5.35 ± 0.15 (n = 24). Adenosine was significantly potentiated by 300 nM NBTI ($_pD_2 = 5.91 \pm 0.38$, $P < 0.01$, n = 6). NBTI (30 μM) produced no further potentiation. The adenosine analogue 5'-N-ethylcarboxamidoadenosine (NECA) when added non-cumulatively produced relaxation ($_pD_2 = 6.64 \pm 0.12$, n = 24). In the presence of NBTI (300 nM), increasing concentrations of the P_1-purinoceptor antagonist 8-sulphophenyltheophylline (8-SPT) (Gustafsson, 1984) produced parallel rightward shifts in the NECA log concentration response curve (Schild plot $_pA_2$ of 5.56 ± 0.11, n = 6, with the slope constrained to 1). Under the same conditions adenosine was also antagonised by 8-SPT (Schild plot $_pA_2$ of 5.54 ± 0.10, n = 6, with the slope constrained to 1). The mean $_pA_2$ of 8-SPT against adenosine was not significantly different from that against NECA ($P > 0.05$).

The low potency of adenosine on cumulative vs non-cumulative addition suggests that rapid desensitisation occurs for events mediated via the P_1- purinoceptor. However , when non-cumulative concentration-effect curves were constructed to adenosine or NECA, in the presence of NBTI, the $_pA_2$ values for 8-SPT against these agonists were similar to those determined in guinea pig aorta, atria and ileum for 8-SPT against 2-chloroadenosine (Collis et al, 1987; Gustafsson, 1984).

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153P MODULATION OF BRADYKININ STIMULATION OF ION TRANSPORT BY GUINEA-PIG GALLBLADDER

M. Woods & A.W. Baird, Department of Pharmacology, University College Dublin, Dublin 4, Ireland.

Bradykinin (BK) contracts smooth muscle (Woods and Baird, 1991) and also causes electrogenic secretion of bicarbonate ions across epithelia (Baird and Margolius, 1989) obtained from guinea pig gallbladder. Each of these pharmacological effects is reversibly abolished by pretreatment with cyclooxygenase inhibitors although pretreatment with subthreshold concentrations of prostaglandin E_2 in the presence of piroxicam could restore the capacity of gallbladder smooth muscle to respond to BK. The aim of this study was to determine whether exogenous application of prostaglandins can influence the sensitivity of the ion transport response to kinins under conditions where endogenous synthesis of prostaglandins is inhibited by the cyclooxygenase inhibitor piroxicam.

Sheets of gallbladder obtained from Dunkin-Hartley guinea pigs were mounted in Ussing chambers (window area=0.63cm^2) and bathed in oxygenated Krebs-Henseleit solution at 37°C. Tissues were voltage clamped by continuous application of short circuit current (SCC). Drugs were added basolaterally to the serosal bathing solution. All experiments were carried out in the presence of captopril (10μM) to avoid any influence of endogenous peptidases on the SCC responses to BK (Woods and Baird, 1992). Results are expressed as mean± s.e.mean. Statistical analysis was carried out using Student's paired t-test or analysis of variance.

BK (10-300nM) added serosally produced a concentration dependent increase in inward SCC (max. response = 47.8±5.8μA; n=5). Piroxicam (1μM; n=7), which reduced the baseline SCC from 12.6 ± 2.2 μA to 3.1 ± 1.7 μA, also reduced the maximal response to 300nM BK by 90.4±2.1% ($p<0.001$) and this action of piroxicam was reversible. PGE_2 (EC_{50}=10.1±1.3nM) stimulated an increase in SCC in a concentration dependent manner. The action of PGE2 was not altered by pretreatment with piroxicam. In the presence of piroxicam, threshold concentrations of PGE_2 (1nM and 3nM) partially restored the capacity of tissues to respond to BK (300nM) which produced an increased SCC of 12.3± 2.3μA and 23.7±5.8μA respectively. Forskolin (500nM), which had no effect on SCC also restored sensitivity to 300nM BK (21.9±2.7μA; n=6).

Our results suggest that PGE_2 acts as a modulator rather than a mediator of BK action in this preparation.When endogenous eicosanoid synthesis is blocked by piroxicam there is a reduction in basal SCC and reduction in tissue sensitivity to kinins. Subthreshold concentrations of PGE_2 are sufficient to partially restore responses to BK. Forskolin also restored SCC responses to BK which suggests that intracellular cyclic AMP may contribute to the complete expression of epithelial ion transport responses in guinea pig gallbladder.

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154P THE EFFECTS OF BRADYKININ AND RELATED COMPOUNDS ON ISOLATED HUMAN MYOMETRIUM FROM PREGNANT AND NON-PREGNANT DONORS

F. Abbas, K. Marshall & J. Senior, Postgraduate Studies in Pharmacology, School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP

Previous preliminary studies in this laboratory have shown that bradykinin (BK) produces a biphasic response on isolated human myometrium (Massele & Senior, 1981). The aim of this study was to investigate further the BK response on human myometrium, from pregnant (P) and non-pregnant (NP) donors, using BK, Lys-BK, des-Arg^9-BK (±2.79μM indomethacin added 1h before experimentation).

Samples of human myometrium were obtained from pre-menopausal patients at hysterectomy or from pregnant patients during Caesarean section (all patients gave written consent). The myometrial strips were set up for superfusion using Krebs' solution (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) and ID_4 (inhibitory) values (nmol) (see Senior *et al.*, 1991).

The responses to BK (0.001-50nmol) and Lys-BK (0.001-50nmol) were biphasic and consisted of an increase in myometrial tension which was followed by a period of inhibition of myogenic activity (both components of the responses being dose-related). The ED_1 values for BK were 7 x 10^{-3} and 8 x 10^{-3} and for Lys-BK 1.5 x 10^{-1} and 3 x 10^{-2} (P and NP respectively). The ID_4 values for BK were 9 x 10^{-2} and 3.9 and for Lys-BK 2.5 x 10^{-1} and > 1 x 10^2 (P and NP respectively) (n = 5 in all experiments). In tissues from pregnant subjects the presence of indomethacin had no significant effect on the excitatory response, but the inhibitory component of the response was reduced (ID_4 BK 7 x 10^{-1}, Lys-BK 2). In tissues from non-pregnant subjects the inhibitory response to BK was similarly affected by indomethacin (ID_4 BK 6); in terms of the excitatory response, indomethacin significantly ($P<0.01$-0.001) enhanced the agonist effect at higher doses. The response to des-Arg^9-BK (0.001-50nmol) was monophasic and contractile on both tissues, ED_1, P, 3 x 10^{-2} and NP, 2 x 10^{-2}. The presence of indomethacin in the superfusate had no effect on P tissue responses to des-Arg^9-BK but it reduced the response in NP tissues at higher doses ($P<0.01$-0.001).

Inhibitory responses in P and NP tissues were reduced by the presence of indomethacin but contractile responses were only reduced in NP tissues, suggesting that cyclo-oxygenase products contribute significantly to responses to BK and its analogues. These findings suggest that different cyclo-oxygenase products are produced by P compared to NP tissues and current work involves characterising the prostaglandin mediators and receptors.

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155P L-NAME-ENHANCED SUSCEPTIBILITY TO REPERFUSION-INDUCED VENTRICULAR FIBRILLATION IS PREVENTED BY L-ARGININE IN RAT ISOLATED HEART

Ravinder Pabla & Michael J. Curtis, Cardiovascular Research Laboratories, Pharmacology Group, Division of Biomedical Sciences, King's College, Manresa Road, London SW3 6LX.

Certain biochemicals present in the heart may confer protection to the ischaemic or reperfused myocardium (Förster, 1976). In the present study we have examined whether endothelium-derived nitric oxide (EDNO) may function as an endogenous protectant against arrhythmias elicited by reperfusion, as proposed by Vegh *et al.* (1992).

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, NaH_2PO_4 1.2, $CaCl_2$ 1.4 and glucose 11.1, pH 7.4, 37°C or similar solution modified by addition of 100 μM N^G-nitro-L-arginine-methyl-ester (L-NAME), 100 μM L-NAME plus 1 mM L-arginine (L-Arg), 100 μM L-NAME plus 10 mM L-Arg or 10 mM L-Arg alone. Hearts (n=100) were randomised to treatment and analysis was blinded. The incidence (out of n=20/group) of reperfusion-induced ventricular fibrillation (VF) was recorded (unipolar electrogram) during 30 min of reperfusion following 60 min of left regional ischaemia. Coronary flow ($ml.min^{-1}.g^{-1}$) in the uninvolved zone (UZ) after 59 min of ischaemia (UZ flow) and recovery of flow ($ml.min^{-1}.g^{-1}$) in the reperfused zone (RZ) after 1 min of reperfusion (RZ flow) were determined by timed collection of coronary effluent. RZ size (RZ%) was determined by the disulphine blue dye exclusion method (Curtis & Hearse, 1989) and expressed as a % of total ventricular weight. RR interval (msec) and QT interval at 100% repolarisation (msec) were determined after 59 min of ischaemia. Results were analysed by Dunnett's test for mean ± s.e.mean data or Chi^2 for % incidence data (Curtis & Hearse, 1989).

Table 1

*(*P<0.05 versus control)*	VF	UZ flow	RZ flow	RZ%	RR	QT
Control	1/20	6.0±0.6	6.9±1.3	41±1	299±18	80±4
100 μM L-NAME	*7/20	*3.4±0.5	4.9±0.9	42±1	*452±55	97±13
100 μM L-NAME + 1 mM L-Arg	2/20	*4.0±0.4	5.1±1.0	41±1	364±63	84±6
100 μM L-NAME +10 mM L-Arg	4/20	5.5±0.5	4.7±1.2	41±1	350±35	78±6
10 mM L-Arg	2/20	*8.6±0.6	7.7±1.1	41±1	275±18	80±4

The incidence of reperfusion induced VF was increased significantly by L-NAME (Table 1). L-NAME caused bradycardia and reduced UZ flow but did not affect RZ size or QT interval (Table 1). All effects of L-NAME were prevented by co-perfusion with 10 mM L-Arg, and all but the reduction in UZ flow were prevented by 1 mM L-Arg. These data are consistent with the hypothesis that endogenous EDNO production protects the heart against reperfusion-induced VF after a sustained (60 min) period of ischaemia in rat.

(R. Pabla is a recipient of a Wellcome Prize Studentship from the Wellcome Trust)

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156P ROLE OF ENDOGENOUS TUMOUR NECROSIS FACTOR, INTERLEUKIN-1 AND PLATELET-ACTIVATING FACTOR IN THE VASCULAR HYPOREACTIVITY IN RATS TREATED WITH ENDOTOXIN

C.C. Wu, Cs. Szabó, C. Thiemermann & J. R. Vane, The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London, EC1M 6BQ, United Kingdom

Endotoxin (lipopolysaccharide, LPS) causes a hyporeactivity of the rat thoracic aorta to noradrenaline (NA) which is due to an induction of nitric oxide (NO) synthase in the vascular smooth muscle (Julou-Schaeffer et al., 1990; Rees et al., 1990). The present study investigates the role of tumour necrosis factor (TNF), interleukin-1 (IL-1) or platelet-activating factor (PAF) in the hyporeactivity of the rat thoracic aorta to NA induced by LPS. Endothelium-denuded rings of thoracic aortas were prepared from rats treated with LPS (2 mg/kg or 10 mg/kg, i.v.) for 3 h. Contractile responses to NA were attenuated as compared to controls (Fig. 1). The development of the hyporeactivity to NA was partially prevented by pretreatment of rats with either a monoclonal TNF antibody (TNFab, raised in hamster, TN3.19.12, Celltech, Slough, UK; 20 mg/kg, s.c. at 16 h prior to LPS), an IL-1 receptor antagonist (IL-1ra, Fisher et al., 1992; 16 mg/kg, i.v. at 15 min prior to LPS followed by 2.4 mg/kg/h for 3 h), a PAF antagonist (WEB 2086, Casals-Stenzel 1987; 5 mg/kg, i.v. at 20 min prior to LPS), or dexamethasone (DEXA, 3 mg/kg, i.v. at 1 h prior to LPS). Contractions to NA were substantially enhanced in rings from LPS treated rats by the NO synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME, 0.3 mM for 20 min). In contrast, L-NAME only slightly enhanced contractions to NA in rings from rats treated with LPS plus IL-1ra (n=8; Fig.1c-d), WEB 2086 (n=8; Fig.1a-b), or DEXA (n=8; Fig.1a-b). However, there was no enhancement to NA by L-NAME in rings from rats given LPS plus TNFab (n=8; Fig.1c-d). Furthermore, in rings from rats given LPS plus WEB 2086 or DEXA the reactivity of contraction to NA was restored by L-NAME to that in normal rings. These results indicate that LPS induces the release of PAF, TNF, and IL-1 *in vivo*, which in turn cause (acting in parallel or in synergy) the induction of NO synthase in the vascular smooth muscle, resulting in the hyporeactivity of vascular smooth muscle in endotoxaemia. *This work was supported by a grant of Glaxo Group Research Ltd. C.C.Wu is supported by the NDMC, Taiwan, ROC. C.Szabó is supported by the Lloyds of London Tercentenary Foundation.*

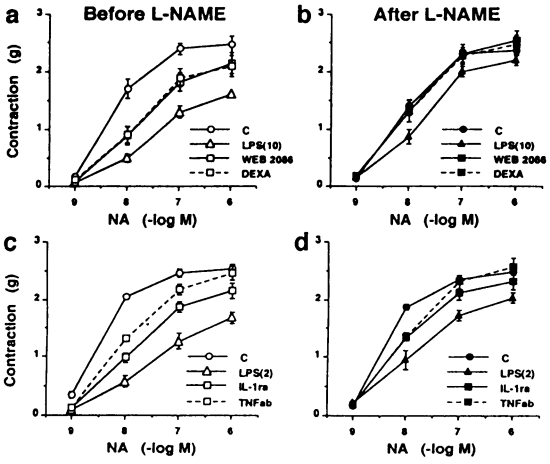

Fig. 1 Effects of WEB 2086 and DEXA (a-b) or TNFab and and IL-1ra (c-d) on the LPS-induced hyporeactivity of the rat thoracic aorta to NA *ex vivo* and the effect of L-NAME *in vitro*

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157P EFFECTS OF N^{ω}-NITRO-L-ARGININE AND METHYLENE BLUE ON NON-ADRENERGIC, NON-CHOLINERGIC RESPONSES OF ISOLATED GUINEA-PIG TAENIA CAECI

W. Piotrowski, M.C. Simon & L. Brennan, School of Applied Physiology, NESCOT, Epsom, KT17 3DS

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). Although the enteric nervous system generally shows a strong NOS immunoreactivity (Bredt, Hwang & Snyder, 1990), non-adrenergic, non-cholinergic (NANC) responses of the isolated guinea pig taenia caeci are reported to be resistant to the effects of the NOS inhibitor L-N^{G}-monomethyl-L-arginine (Rand & Li, 1990). It has recently been shown that approximately one third of cultured neurones taken from the myenteric plexus of this tissue stain NOS immunopositive, strongly suggesting a role for NO in this preparation (Saffrey *et al*, 1992). The purpose of the present study was to examine further the possible involvement of NO in NANC responses in the taenia caeci.

Segments of taenia caeci 2cm long were removed from freshly killed guinea pigs (450-780g) 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%O_2/5%CO_2. Supramaximal electrical stimulation using 1ms rectangular pulses was applied in trains lasting 10s and repeated every 90s. Contractile activity of the preparations was recorded isotonically using a resting tension of 0.5g.

Under these conditions, the NOS inhibitor N^{ω}-nitro-L-arginine (NOARG)(Moore *et al*, 1989)(10μM), produced an inhibition of electrically-evoked NANC relaxations. The effect of NOARG appeared to be greater at the higher stimulation frequencies tested: (means$\pm$sem, control relaxations=100%: 5Hz 91.2$\pm$6.4%; 10Hz 81.3$\pm$2.2%*; 20Hz 81.4$\pm$3.3%*)(n=6). The effects of NOARG at 20Hz were partially reversed with L-arginine (1mM)(90.0$\pm$2.1%‡). NANC relaxations evoked by stimulation at 20Hz appeared to be inhibited by NOARG in a concentration-dependent manner, with relaxations of 54.1$\pm$4.4% corresponding to a maximal effect and an IC_{50} for NOARG of 4.5$\pm$1.8μM (n=6). Methylene blue (30μM), an inhibitor of soluble guanylyl cyclase, appeared to have little effect on the NANC relaxations produced by stimulation at 20Hz. (95.6$\pm$1.8%, n=6). To these same tissues, NOARG (10μM), added subsequently, was still able to produce an inhibition of the observed relaxations (68.7$\pm$6.2%*, n=6).

The results suggest that NO may indeed have a role in mediating a component of the electrically-evoked NANC relaxations of the guinea pig taenia caeci, and that NO may be producing its effects via a mechanism which does not involve soluble guanylyl cyclase. (*=$p<0.05$, compared to pre-drug controls, ‡=$p<0.05$ compared to responses in presence of NOARG, Student's t-test)

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158P ROLE OF SPHINGOMYELIN METABOLISM IN SUPEROXIDE GENERATION BY TNFα IN HUMAN NEUTROPHILS

F. Yanaga and S.P.Watson, Department of Pharmacology, Oxford University, Mansfield Road, Oxford, OX1 3QT

Tumor necrosis factor α (TNFα) stimulates superoxide generation in neutrophils and, at lower concentrations, primes neutrophils for superoxide generation in response to other stimuli (e.g. fMLP). Recently, TNFα has been shown to activate a neutral sphingomyelinase to generate ceramide, and it has been suggested that ceramide may be a second messenger mediating the effect of TNFα (Obeid *et al*, 1993). We have therefore examined the effect of TNFα on sphigomyelin hydrolysis in human neutrophils and have investigated the action of sphingomyelinase and C2-ceramide, a cell permeable analogue of ceramide, on superoxide generation in this cell type.

The effect of TNFα on superoxide generation was investigated using the nitro blue tetrazolium (NBT) reduction assay. TNFα stiumulates superoxide generation in a concentration- and time-dependent fashion. The maximally effective concentration of TNFα for superoxide generation was 10 nM and maximal increases were obtained after 20 min. Two major types of receptor for TNFα with apparent molecular masses of 75 kDa and 55 kDa have been identified. To clarify wherther one or both types of receptor mediate stimulation of superoxide generation, we used two monoclonal antibodies, utr-1 and htr-9. Utr-1 behaves as an antagonist at the 75 kDa receptor and htr-9 behaves as an agonist at the 55 kDa receptor. Utr-1 (5μg/ml) inhibited the effect of TNFα to 57.1±3.7 % (n=6) of the control response while htr-9 (5μg/ml) mimicked the effect of TNFα but with a lower maximal response (78.8±4.1 %; n=6).

Ceramide is converted to ceramide 1-phosphate by ceramide kinase (Dresseler et al, 1990) and we have measured this metabolism to clarify the effect of TNFα on sphingomyelinase activity in neutrophils. Although exogenous sphingomyelinase (1mU/ml) or C2-ceramide (10 μM) increased the amount of ceramide 1-phosphate 3.4 fold ($p<0.01$) and C2-ceramide phospate 10.3 fold ($p<0.01$), respectively, in a time dependent manner, TNFα (10 nM) had no effect on the level of ceramide 1-phosphate. Neither exogenous sphingomyelinase or the cell permeable analogue of ceramide, C2-ceramide, stimulated production of superoxide or induced priming in response to fMLP.

These results suggest that TNFα stimulates superoxide generation through both the 55 kDa and 75 kDa receptors but that ceramide does not act as second messenger in this response.

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159P VITAMIN E OFFERS PROTECTION AGAINST THE IMPAIRMENT OF ENDOTHELIUM-MEDIATED RELAXATION IN THE CHOLESTEROL FED RABBIT AORTA BUT NOT IN THE HIND LIMB

G.R. Shaw, J.R. Keddie, J. Pinkney, P.N. Clarkson, J.W. Growcott & S.M. Poucher, Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG

Hypercholesterolaemia has been shown to induce endothelial dysfunction (Girerd et al 1990), leading to endothelium-dependent relaxant responses being impaired. This is most probably a result of an attenuation in the production or release of endothelium derived relaxing factor or its increased breakdown. Vitamin E has been shown to protect against impairment of endothelium-mediated relaxation in the cholesterol-fed rabbit *in vitro* (Stewart-Lee et al 1992). This study was designed to investigate the protection of the endothelium offered by vitamin E *in vivo* as well as *in vitro*. Male N.Z. White rabbits (2.5 - 3.4 kg) were fed either: a) normal rabbit chow (n=11), b) rabbit chow with a 2% cholesterol supplement (n=5) or c) b plus 2100 iu.week^{-1} vitamin E (n=8) for 8 weeks. The rabbits were anaesthetised using a combination of Hypnorm and Hypnovel. A double lumen catheter was inserted into the left femoral artery and fed proximally to the bifurcation of the abdominal aorta. This catheter was used for monitoring hind limb blood pressure and for the administration of acetylcholine (ACh). Right femoral artery blood flow was monitored using a 2.5 mm circumference electromagnetic flow probe. Conductance changes (femoral blood flow/hind limb blood pressure) to dose response curves (DRCs) to ACh were investigated. The cholesterol group showed an attenuated endothelium-dependent vasodilatation, the DRC to ACh was shifted 11.1 fold rightward from the normal group's response supporting the findings of Verbeuren et al (1986) that hypercholesterolaemia impairs endothelium-mediated relaxant responses in isolated rabbit arteries.

The cholesterol + vitamin E group produced ACh DRCs which were indistinguishable from the cholesterol alone group. However *in vitro* studies performed on tissues from these same animals produced contrasting results. ACh DRCs were performed on aorta which had been pre-constricted with a maximal dose of 5-HT. The maximum relaxation from the normal, cholesterol and cholesterol + vitamin E groups were (n=4) 68.2±3.7, (n=5) 35.8±3.2 and (n=4) 55.8±3.3 % relaxation respectively. The relaxation of the cholesterol alone group was statistically different from the normal and cholesterol + vitamin E groups ($p<0.001$ and $P<0.01$ respectively), whilst the cholesterol + vitamin E group was still significantly different from the normal group ($p<0.05$). It would therefore appear that vitamin E diet supplementation ameliorates the deleterious effects of a high cholesterol diet in large vessels such as the aorta but, the smaller resistance vessels of the rabbit hind limb are offered no discernible protection against impaired endothelium-mediated relaxation by vitamin E.

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160P THE EFFECT OF COLCHICINE ON IP PROSTANOID RECEPTOR DESENSITIZATION IN NG108-15 CELLS

A Krane and M Keen, Department of Pharmacology, The Medical School, University of Birmingham, Birmingham B15 2TT, UK

Colchicine, an inhibitor of microtubule polymerization, has been reported to inhibit desensitization produced by pretreating a number of cell types with ß-adrenoreceptor agonists or prostaglandin E_1 (Harden, 1983). In this study we have examined the effects of colchicine on iloprost mediated desensitization in NG108-15 cells.

NG108-15 cells (passage 16-25) were grown to confluency in 80cm^2 flasks. Cells were pretreated with 10μM iloprost or vehicle in Dulbecco's modified Eagle's medium for 17h at 37^{o}C, in the presence or absence of 1μg/ml colchicine. Cells were then harvested and washed three times in phosphate buffered saline and frozen at -70^{o}C until required. Adenylate cyclase activity and the specific binding of [^{3}H]-iloprost were assayed in cell homogenates as previously described (Kelly et al., 1990), except that binding assays were performed in the presence of 100μM 5'guanylylimidodiphosphate to identify receptors uncoupled from G protein. Data were compared using paired t-tests, with $P<0.05$ taken to be significant.
Colchicine produced a significant reduction in the loss of [^{3}H]-iloprost binding activity produced by iloprost pretreatment. Pretreatment of NG108-15 cells with iloprost in the absence of colchicine led to an 80% reduction in the specific binding of 11.3 nM [^{3}H]-iloprost, from 28.3±4.5 fmol/mg protein in membranes from control cells to 5.1±5.3 fmol/mg protein in membranes iloprost treated cells. In the presence of colchicine the reduction in specific binding produced by iloprost pretreatment was 45%, being 28.5±7.3 and 15.8±2.9 fmol/mg protein in membranes from control and iloprost treated cells, respectively. (All values mean±s.e.mean, n=4). Colchicine also appeared able to attenuate the decrease in the ability of 1μM iloprost, 10mM NaF and 100μM N-ethylcarboxamidoadenosine (NECA) to stimulate adenylate cyclase activity which is produced by iloprost pretreatment. Following pretreatment in the absence of colchicine, the response to iloprost was reduced by 70%, from 77.3±37.6 to 22.6±9.6 pmol cAMP.min-1.mg protein-1. In the presence of colchicine the response to iloprost was reduced by 40%, from 38.7±25.2 to 22.7±13.2 pmol cAMP.min-1.mg protein-1. In the absence of colchicine, the response to NECA was reduced by 30%, from 25.6±7.3 to 17.9±7.9 pmol cAMP.min-1.mg protein-1; in the presence of colchicine this response was reduced by only 5%, from 17.9±7.3 to 17.1±8.4 pmol cAMP.min-1.mg protein-1. In the absence of colchicine, the response to NaF was reduced by 45%, from 36.2±15.6 to 19.2±7.4 pmol cAMP.min-1.mg protein-1; in the presence of colchine this response was not reduced, being 15.8±7.3 and 17.1±10.4 pmol cAMP.min-1.mg protein-1 in membranes from control and iloprost treated cells, respectively. (All values mean±s.e.mean, n=5).

Colchicine significantly reduced the loss of [^{3}H]-iloprost binding activity produced following pretreatment with iloprost and also appeared to reduce functional desensitization. However, inhibition of desensitization occured as a result of a decrease in control responses, there being little effect of colchicine on the responses observed following iloprost pretreatment. It remains to be established whether the effects of colchicine are due to inhibition of a process of desensitization or due to a less specific impairment of receptor and/or G protein function.

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161P AGE-RELATED CHANGES IN POTENCY OF ENDOTHELIN-1 AND ENDOTHELIN-3 OCCUR IN THE RAT AORTA BUT NOT IN THE TRACHEA

M. Kengatharan, T.J. Brown, D. Raeburn and A. G. Roach. Rhône-Poulenc Rorer, Dagenham Research Centre, Rainham Road South, Essex, RM10 7XS.

Endothelin-1 (ET-1) is a potent contractile agent of isolated vascular smooth muscle and airways tissue (Sakurai *et al*, 1992). Although the effects of aging on the potency of ET-1 has been studied in rat aorta (RA; Ishihata *et al*, 1991), we have investigated the influence of age on the potency (pD_2) of ET-1 and endothelin-3 (ET-3) in the RA and in the rat trachea (RT).

Rings of RA and RT (3mm) were removed simultaneously from male albino Wistar rats. The endothelium was physically removed from RA. The rings were suspended under resting load of 2g (RA) and 1g (RT) in 5ml siliconised organ baths containing Krebs' solution gassed with 95%O_2:5%CO_2. Following equilibration for 1h, reproducible responses to phenylephrine (100nM, RA) and to carbachol (300nM, RT) were obtained to provide reference responses. L-nitroarginine (100μM) was added to baths containing RA to ensure abolition of nitric oxide synthesis. Contractile responses to ET-1 and ET-3 were measured using standard techniques and standardised to the reference response. Since the age of the rats could not be estimated accurately the weight of the rats were used as an index of age.

TABLE 1. Potency of ET-1 and ET-3 on rat thoracic aorta and trachea. Experiments relating to specific weight range of animals were run in parallel.

		RAT AORTA			RAT TRACHEA		
Group	Rat weight (g)	ET-1 pD_2	ET-3 pD_2	Relative Potency [ET-1/ET-3]	ET-1 pD_2	ET-3 pD_2	Relative Potency [ET-1/ET-3]
A	146±2	8.56±0.08	7.80±0.14	6.1±1.1	7.32±0.09	7.34±0.17	1.3±0.5
B	257±6	8.38±0.05	7.57±0.08	6.8±1.4	7.22±0.07	7.36±0.07	0.8±0.1
C	298±3	8.57±0.03	7.00±0.08**	37.6±4.8#	7.23±0.07	7.33±0.07	1.0±0.2
D	350±3	8.26±0.09*	6.50±0.03**	59.7±8.9#	7.44±0.04	7.50±0.09	0.9±0.1
E	528±6	8.14±0.08*	6.43±0.11**	52.8±4.8#	7.47±0.05	7.40±0.15	1.2±0.2

(values are given as mean ± s.e. mean, n=4; analysis of variance followed by Dunnett's method *$p<0.05$, **, #$p<0.01$ compared to group A).

These data indicate that the potency of ET-1 in RA declines with increasing age (above 300g), a finding consistent with that reported by Ishihata *et al* (1991). However, a larger decline in potency of ET-3 compared to ET-1 was noted in this tissue as shown by a significant increase in potency ratio with aging. In contrast, the potencies of ET-1 and ET-3 in RT are not influenced by age. On the basis of order of agonist potency (Sakurai *et al*, 1992) our data indicate that ET_A receptors (order of potency ET-1>ET-3) and ET_B receptors (order of potency ET-1=ET-3) mediate contraction to endothelins in RA and RT respectively. In conclusion, it seems likely that the ET_A receptor mediated-responses become less sensitive whereas the ET_B mediated-responses remain unchanged with increasing age of rats. Additionally, in the light of the finding that the potency of ET-3 in the RA decreased significantly more than ET-1 may indicate multiple subtypes of ET_A receptors in this tissue.

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162P SELECTIVE ENHANCEMENT OF MYOFIBROBLAST-ACTIVATED CONTRACTILITY IN PARAQUAT PRETREATED RAT LUNG TISSUE

R. Hicks & A.A. Hemati, Postgraduate Studies in Pharmacology, University of Bradford, Richmond Road, Bradford West Yorkshire, BD7 1DP

In numerous tissues, contractility to certain characteristic agents is associated with cells other than muscle, e.g. actin-containing so-called myofibroblasts. Such cells are present in pulmonary interstitium of several mammalian species (Kapanci *et al.*, 1974) but their contractility has only been demonstrated from the proliferation of myofibroblasts in fibrotic lung tissues (Hicks *et al.*, 1991). This study was to evaluate such responses in normal tissues, in contrast with fibrotic lungs and it was necessary to take account also of smooth muscle reactivity. Distinctive agonists: mepyramine (MEP) and sodium tungstate (ST) characterised myofibroblast mediated contractility, in contrast with acetylcholine (ACH) and barium chloride(BC) as specific smooth muscle stimulants.

Lung tissue strip preparations were obtained from either normal, female Sprague Dawley rats (200-250gm) or animals in which pneumonitic or fibrotic changes were induced by paraquat (single, oral doses, 10mg/kg). Lungs removed from normal animals or at 1 to 6 weeks after treatment, were cut to give longitudinal strips (2.5 x 2 x 20mm). Weighed preparations, suspended in gassed Krebs solution at 37°C were equilibrated to resting tensions of 1gm for 90 minutes. Contractions, measured isometrically, were standardised in terms of tissue lengths and weights.

Ascending sequences of doses of MEP (1 x 10^{-5} to 5 x 10^{-3}M), ST (1 x 10^{-5} to 5 x 10^{-3}M), BC (1 x 10^{-6} to 5 x 10^{-3}M) or ACH (1 x 10^{-6} to 2 x 10^{-3}M) induced slow, sustained contractions, dose-related and repeatable, in both normal (n = 12) or pretreated tissues (n = 10), each response reversible on washing. Responses to MEP or ST were significantly higher in paraquat treated tissues than in normal lung, especially at 1 week, as exemplified by comparing submaximal responses to 1mM concentrations (Figure 1). Responses to ACH or BC diminished with time in the treated lungs.

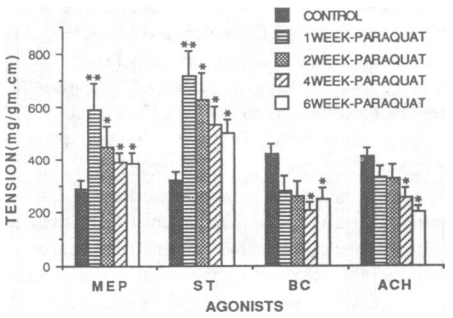

Figure 1. Contractile responses to representative submaximal doses (1mM) of test agonists in parenchymal strips from lungs of normal (control) or paraquat-pretreated rats.

**Significantly different from control ($P<0.01$), *($P<0.05$).

Contractility to MEP and ST in normal lungs is attributable to myofibroblasts. Early enhancement of reactivity due to paraquat may be due to myofibroblast proliferation, declining with fibrotic development. Smooth-muscle contractility to ACH and BC may decline due to replacement of tissue.

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163P RELAXANT EFFECTS OF CYCLIC GMP-ELEVATING AGENTS ON SPASMOGEN-SUPPORTED TONE IN BOVINE TRACHEAL SMOOTH MUSCLE

Samuel C. Ijioma, R.A. John Challiss and John P. Boyle+, Departments of Pharmacology and Therapeutics, and +Physiology, University of Leicester, Leicester LE1 9HN.

Relaxation of airway smooth muscle by agents which increase cyclic AMP concentration has been extensively studied and much is known about the biochemical mechanisms which are responsible for this therapeutically important action (see Giembycz & Raeburn, 1991). In contrast, although it has been realized for sometime that elevation of airway smooth muscle cyclic GMP content can also cause relaxation (Katsuki & Murad, 1977), much less is known about the mechanisms underlying this functional effect. Here, we have investigated the effects of sodium nitroprusside (SNP) and atrial natriuretic peptide (ANP; rat 3-28), which respectively activate soluble and particulate forms of guanylyl cyclase, in bovine tracheal smooth muscle (BTSM).

Trachea were dissected free of epithelium and connective tissue in ice-cold Krebs-Henseleit buffer (KHB). BTSM was cut into strips (10 x 2 x 2 mm) and mounted in KHB at 37°C for the isometric measurement of tension. Alternatively, tissue was cross-chopped (300 x 300 μm), washed extensively in KHB and cyclic GMP responses to SNP or ANP assessed as described previously (Challiss et al., 1992).

The spasmolytic effects of SNP and ANP were compared to the response to isoprenaline (ISO) in BTSM strips where tone was induced by either 100 μM histamine (HIST) or 0.1 μM methacholine (MCh). SNP, ANP and ISO each produced concentration-dependent relaxations of HIST-supported tone (EC_{50}s 320 ± 80; 2.8 ± 0.8 and 6.6 ± 4.3 nM respectively; mean ± s.e.m. (n=6)), with the maximal relaxations evoked by SNP and ANP being about 80% of that induced by isoprenaline (ISO_{max}). SNP was also an effective relaxant of MCh-contracted BTSM (EC_{50} 290 ± 90 nM compared to that for ISO of 30 ± 7 nM (n=8 for each)), again causing a maximal relaxation which was 78% of the ISO_{max}. In contrast, ANP caused only a 14.1 ± 5.5% relaxation of MCh-contracted BTSM, relative to the ISO_{max}, at a concentration (300 nM) which was maximally effective against HIST-supported tone. SNP caused a concentration-dependent increase in cyclic GMP in BTSM slices (EC_{50} 40 ± 2 μM) which amounted to a 10-fold increase over basal levels at 300 μM. ANP (300 nM) caused a 6-fold increase in cyclic GMP (basal, 0.45 ± 0.06; +ANP, 3.11 ± 0.88 pmol/mg protein (n=4)). Neither SNP nor ANP affected BTSM cyclic AMP levels.

These data suggest that stimulation of either soluble or particulate guanylyl cyclase can increase cellular cyclic GMP concentration and effect relaxation of HIST-supported tone in BTSM. However, ANP-receptor/guanylyl cyclase activation appears to be much less effective in causing relaxation of BTSM pre-contracted with MCh, compared to that evoked by the soluble guanylyl cyclase activator SNP.

We thank the British Lung Foundation and Leicestershire Health Authority for financial support.

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164P ANTIGEN-INDUCED CONTRACTION OF GUINEA-PIG TRACHEA AND PARENCHYMA: EFFECT OF THE 5-LIPOXYGENASE INHIBITOR BW B70C AND MODULATION BY INDOMETHACIN AND MEPYRAMINE

M. Yeadon, H. C. W. Jones & A. N. Payne, Department of Pharmacology, Wellcome Foundation Ltd., Beckenham, Kent, BR3 3BS.

It is widely believed that leukotrienes, products of the 5-lipoxygenase (5-LO) pathway, and histamine are the principal agents mediating antigen-induced contractile responses of animal (Cho, *et al.* 1991) and human airway tissue (Bjorck & Dahlen, 1993). However, most of the published work in this area has focussed on the upper airways. Since the contribution of upper and lower airways narrowing to airflow limitation in allergic asthma may vary, we have compared the effect of the potent 5-LO inhibitor BW B70C (Payne, *et al.* 1991) on antigen-induced contraction of both isolated tracheal and lung parenchymal strips from guinea-pigs.

Guinea-pigs (300-450g) were actively sensitised to ovalbumin (OVA; 50mg s.c. and i.p.) and 14-28d later, tracheal and parenchymal lung strips were mounted in organ baths (25ml Krebs', 37°C, 95%O_2/5%CO_2) under 2g or 1g tension respectively in the presence or absence of indomethacin (I; 5μM) and/or mepyramine (M; 10μM). After 60min equilibration, tissue viability was assessed by eliciting a contraction to ACh (100μM). Basal tone was re-established 60min later, and BW B70C (0.03 - 30μM) or vehicle (5μl DMSO) was added, and after a 15min incubation, the tissues were contracted with OVA added cumulatively (0.01 - 1000ng ml^{-1}). Responses were calculated as the percentage of the contraction to ACh. IC_{50} values for BW B70C were calculated by interpolation at the concentration of OVA giving maximum response.

In trachea, the combination of I and M enhanced the maximum contractile responses to OVA by 63% ($P<0.001$). Paradoxically, neither I nor M alone had any effect on maximum response but either alone produced >50% inhibition of response at low concentrations of antigen. BW B70C inhibited OVA-induced contractions in trachea with similar potency in the presence (IC_{50}=0.66μM: 80±5% inhibition at 1μM BW B70C) and absence (IC_{50}=0.88μM: 68±10% inhibition at 1μM BW B70C) of I and M.

In parenchymal strips, concentrations of BW B70C (1μM) which in trachea produced substantial (>50%) inhibition of responses to OVA had no effect on contractile responses to OVA in the absence of I and M. Even at high concentrations (30μM BW B70C), inhibition of maximum responses was <50%. Neither I nor M alone significantly altered responses of parenchyma to OVA. The combination of I and M had no effect on maximum responses to OVA, but resulted in a small (half-log) rightward shift in the position of the OVA-response curve. In the presence of I and M, BW B70C displayed a similar potency as in trachea, with an IC_{50} of 0.46μM (53±17% inhibition at 1μM). Interestingly, this facilitating effect of pretreatment with both I and M was seen when either drug was used alone in combination with 1μM BW B70C.

This study suggests that in guinea-pig lower, but not upper, airways, cyclo-oxygenase products and histamine may facilitate release of or responses to leukotrienes elaborated after OVA stimulation. The striking effect of I or M in parenchymal, but not tracheal, strips on the inhibitory effect of a 5-LO inhibitor against antigen-induced contraction calls for a parallel investigation in human airways *in vitro*.

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165P SUBCUTANEOUS SILICONE IMPLANTS IN THE RAT: A MODEL FOR CAPSULAR CONTRACTURE?

C.M. Malata, I.L. Naylor* & M.J. Timmons, * Postgraduate School of Studies in Pharmacology and Plastic Surgery & Burns Research Unit, University of Bradford, Richmond Road, Bradford, West Yorks, BD7 1DP

Capsule formation and its subsequent contraction around implanted devices remains an unresolved problem in plastic surgery despite attempts to modify it by procedures ranging from alterations of the surface structure of the implanted device (Coleman et al., 1991) to the local administration of steroids (Peterson and Burt, 1974). This communication describes an animal model which may facilitate the rational development of drugs to either inhibit or reduce the contractile abilities of such capsules.

Female, Hooded Lister rats (230 ±10g, n = 70) had solid silicone rods (10mm diameter, 20mm in length) implanted into the dorsal subcutaneous tissue space. The rods were removed 7, 14, 21, 28, 56 and 84 days after implantation. The exudate around each implant was examined cytologically. The capsules were subjected to optical microscopy and to an in vitro determination of their contractile ability (Coleman et al., 1987). Groups of animals (n = 50) acted as controls (C) whilst others (n = 20) received prednisolone (T), 5mg kg^{-1} for 7 days ip and its effect studied at 7, 28 and 56 days.

Prednisolone reduced exudate volume (mean ± sem mls): C=7(2.4±1.03), 14(0.97±0.88), 21(0.66±0.36), 28(0.1±0.28), 56(0.0), 84(0.0); T= 7(0.49±0.40), 28(0.05±0.03), 56(0.0). Histological analysis showed that the early (day 7) capsule thickness (μm) in control animals was significantly greater ($P<0.05$, Mann Whitney-U test [M W-U test]) than in steroid treated capsules: C=245.08 (194.35-295.83); T=55.55 (43.32-67.78). In vitro the reactivity of the control capsules was maximal at 7 days and then progressively declined: median contractile response to 4mg mepyramine (mg tension/ g of capsule) - 7(40.4), 14(25.9), 21(21.6), 28(15.2), 56(16.0), 84(18.7). At the time of maximal reactivity (day 7) the contractility of treated capsules was significantly less ($p< 0.05$, M W-U test) than controls (T= 16.6, range 8.0-28.2; C= 40.4, range 21.4-103.0).

These results suggest that in the rat, silicone rods can induce capsule formation which can model the process of capsule formation in man and that early capsules can be modified by the use of prednisolone.

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Peterson, H.D. & Burt, G.B. (1974) *Plast. Reconstr. Surg.* 54, 28-31.

166P EFFECT OF GLYCINE ON CISPLATIN-INDUCED ACUTE RENAL FAILURE IN THE RAT

Q. Li, M.S. Yates & C.J. Bowmer, Department of Pharmacology, The University of Leeds, Leeds LS2 9JT.

Infusions of glycine have been shown to ameliorate cisplatin (CP) nephrotoxicity (Heyman *et al.*, 1991). This work aimed to assess the potential of single bolus doses of glycine to prevent renal damage and to reverse any early changes in renal haemodynamics induced by CP. Male Wistar rats received either CP alone (6 mg kg^{-1}, i.v.) or CP followed by glycine: 0.25, 0.5 and 1.0 g kg^{-1}, i.v. A control group received CP plus a dextrose solution (5 ml kg^{-1}) isosmotic with 1 g kg^{-1} of glycine. Renal function was monitored by measuring urine output, and clearances of [^{3}H]-inulin (C_{IN}) and [^{14}C]-p-aminohippurate (C_{PAH}) whilst in acute experiments, renal blood flow (RBF) was measured with an ultrasonic flow probe.

TABLE 1

Group	Normal	CP	CP + Dextrose (5.0 ml kg^{-1})	CP + Glycine (0.25 g kg^{-1})	CP + Glycine (0.50 g kg^{-1})	CP + Glycine (1.0 g kg^{-1})
Urine output (ml $24h^{-1}$)	8 ± 1	35 ± 3	27 ± 2	21 ± 1	15 ± 2**†	14 ± 1**†
C_{IN} (ml min^{-1} $100g^{-1}$)	1.4 ± 0.1	0.30 ± 0.04	0.11 ± 0.01	0.46 ± 0.08*	0.56 ± 0.16**	0.77 ± 0.08***†
C_{PAH} (ml min^{-1} $100g^{-1}$)	2.7 ± 0.2	1.0 ± 0.1	0.76 ± 0.12	2.1 ± 0.2**	2.1 ± 0.3**	2.9 ± 0.2***††

Mean ± s.e. mean (n = 8), measured 8 days after induction of renal failure.
$^{*}P < 0.05$; $^{**}P < 0.01$, $^{***}P < 0.001$ relative to CP + dextrose; $^{\dagger}P < 0.05$ relative to CP + 0.25 g kg^{-1} glycine (ANOVA); $^{\dagger\dagger}P < 0.05$ relative to CP + 0.5 g kg^{-1} glycine (ANOVA).

CP injection resulted in polyuria and falls in C_{IN} and C_{PAH} (Table 1). Treatment of CP-injected rats with bolus doses of glycine attenuated CP-induced renal dysfunction with the largest dose of glycine (1.0 g kg^{-1}) producing the greatest beneficial effect. In anaesthetised rats, CP significantly ($P<0.001$) reduced RBF from 5.5 ± 0.4 to 2.8 ± 0.6 (n = 6) ml min^{-1} over the course of 2h. Treatment of rats with both CP and glycine (1 g kg^{-1}) resulted in no change in RBF (5.6 ± 0.3 before treatments and 5.5 ± 0.3 ml min^{-1}, n=6, 2h later). This protective effect of glycine on RBF in CP-treated rats was partially reversed by infusion of N^{ω}-nitro-L-arginine methyl ester, 10 μg kg^{-1} min^{-1} (RBF 4.7 ± 0.3 before treatments and 3.8 ± 0.2 ml min^{-1}, n=6, 2h later).

The results demonstrate that bolus doses of glycine ameliorate CP-induced acute renal failure and that the amino acid attenuates CP-induced acute falls in RBF, an effect mediated in part by nitric oxide.

Heyman, S.N., Rosen, S., Silva, P., Spokes, K., Egorin, M.J. & Epstein, F.H. (1991) *Kidney Int.* **40**, 273-279

167P TISSUE DISTRIBUTION IN TUMOUR BEARING MICE OF A NEW AGENT (5,10,15,20-TETRA[M-HYDROXYPHENYL]CHLORIN) FOR PHOTODYNAMIC THERAPY OF CANCER

A.T. Michael-Titus, S. Basra, R. Whelpton & M. Grahn[1], Department of Pharmacology, Queen Mary & Westfield College, Mile End Road, London E1 4NS, and [1]The Surgical Unit, London Hospital Medical College, Whitechapel, London, E1 2AD.

Photodynamic therapy (PDT) is a treatment for cancer based on the use of agents which become cytotoxic on exposure to light. Tetra(m-hydroxyphenyl)chlorin (m-THPC) is a new photosensitizer currently undergoing clinical trials. We report its kinetics and tissue distribution in tumour bearing mice.

Female Balb/c mice (18-22g) were injected s.c. in the flank region with a suspension of Colo-26 tumour cells. After two weeks, m-THPC (0.75 μmol/kg) was injected i.v. and the animals killed at 1, 2, 4, 8, 24 and 48 h post dose. Blood and tissue concentrations of m-THPC were determined by HPLC with UV and fluorescence detection (Wang *et al.*, 1993a,b). Blood levels were fitted to a bi-exponential decay model with rate constants ±s.d. of 0.61 ± 0.24 h^{-1} and 0.050 ± 0.008 h^{-1}. Concentrations in liver declined in a similar manner, the terminal rate constant being 0.053 ± 0.013 h^{-1} (Figure 1). The half-lives for the terminal decay in lung (43 h) and brain (60 h) concentrations were longer than those of blood and liver, 14 and 13 h, respectively. Concentrations in skeletal muscle remained almost constant (range 0.27 - 0.33 μg/g) throughout the period of the study. Mean tumour concentrations ±s.e.mean rose from 0.49 ± 0.06 μg/g at 1 h to a maximum of 1.49 ± 0.25 μg/g at 24 h. As the tumour concentration was still high (1.23 ± 0.4 μg/g) at 48 h, this time gave the maximal tumour:tissue ratios. These data support the clinical practice of irradiating tumours 48-72 h post dose; however it cannot be assumed that all tumour types will exhibit the same degree of selectivity.

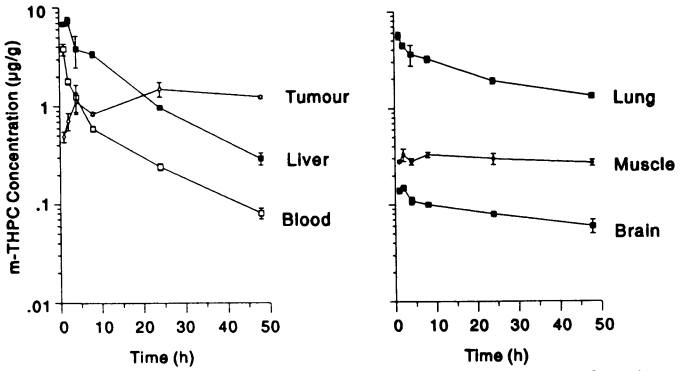

Figure 1. m-THPC concentration (μg/g wet weight) as a function of time. Each point represents the mean ± s.e.mean for 4 or 5 animals.

We are grateful to Scotia Pharmaceuticals Ltd for providing m-THPC.

Wang, Q., Ris, H-B., Altermatt,H.J., Reynolds, B., Stewart, J.C.M., Bonnett, R. & Lim, C.K. (1993a) Biomed. Chromatogr. 7, 45-47.
Wang, Q., Altermatt, H.J., Ris, H-B., Reynolds, B., Stewart, J.C.M., Bonnett, R. & Lim, C.K. (1993b) Biomed. Chromatogr. 7, 155-157.

168P METABOLISM AND PHARMACOKINETICS OF THE NITROSOUREA B.3996

P.M. Loadman, P.B. Hulbert[1] & M.C. Bibby, Clinical Oncology Unit; and [1]Pharmaceutical Chemistry, School of Pharmacy; University of Bradford, Bradford BD7 1DP, UK.

A series of experimental anticancer compounds - in which an alkylating nitrosourea is chemically linked to a 5-fluorouracil (5-FU) moiety - has been designed and synthesised in Dublin by McElhinney, and the chemotherapeutic activity of the series is being determined at Bradford (McElhinney *et al.*,1988). Within this series, compound B.3996 (Figure 1.) shows good activity against a range of transplantable solid mouse adenocarcinomas of the colon (MAC tumours) and therefore B.3996 is being examined further from pharmacokinetic and metabolic viewpoints.

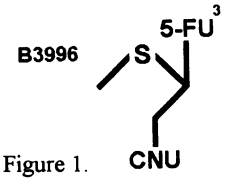

Figure 1.

An HPLC method for the separation of B.3996 and its metabolites from mouse plasma was developed: plasma from treated mice was extracted using C-18 solid phase cartridges and eluted with MeOH. Separation was achieved using a RP-18 column and a MeOH/phosphate buffer (50mM, pH 3) eluent (ratio 32:68), at 1.2 $ml.min^{-1}$ with detection at 265nm. B.3996 (100mg kg^{-1}) was administered either ip or orally, and mice (n = 3)sacrificed at each time point to obtain blood samples by cardiac puncture. An internal standard was added to plasma samples (50-100μl) before extraction.

B.3996 (by ip route) was absorbed rapidly with a max. conc. of 35.4 μg ml^{-1} at 5 min, a half-life of 33 min, and a AUC of 27.1 μg h ml^{-1}. High levels of two metabolites M1 and M2 were observed, and 3 other minor metabolites were also detected. The relative retention times for M1 and M2 were 0.46 and 0.61 (B3996=1.0); their AUCs were 11.5 and 29.2 μg h ml^{-1}. Oral administration showed rapid absorption, a biphasic profile, lower peak plasma levels at 12.2 μg ml^{-1}, a terminal half-life at 107 min, a AUC of 10.0 μg h ml^{-1}, and a bioavailability of only 37%. M1 and M2 were seen at similar levels to that of B.3996 with AUCs of 8.8 and 6.9 μg h ml^{-1}. In separate assays, only very low levels of 5-FU were observed (by either route).

The chemical identities of metabolites M1 and M2 are being elucidated by m.s. M1 arises by oxidative metabolism: it is most likely to be the sulphoxide as it can be obtained by H_2O_2 treatment of B.3996 (the sulphone is one of the minor metabolites). M2 probably arises by *in vivo* lysis of the alkylating moiety of B.3996: it can be obtained by mild alkaline hydrolysis of the N-(2-chloroethyl)-N-nitrosourea (CNU) group of B.3996. M1 is therefore predicted to be an active metabolite, and M2 an inactive metabolite. As 5-FU is only a very minor metabolite, the chemotherapeutic activity of B.3996 arises predominantly from the CNU alkylating group; the 5-FU group (which is linked to the rest of the molecule through its N-3 atom) may play a role in active drug transport.

Precise identification and chemotherapeutic testing of the metabolites is necessary to assess their contribution to the activity/toxicity pattern of this and other members of the series.

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169P PLASMA PHARMACOKINETIC STUDIES ON B.3970, A MOLECULAR COMBINATION OF 5-FLUOROURACIL AND N-(2-CHLOROETHYL)-N-NITROSOUREA (CNU), IN A MURINE MODEL

P.M. Loadman, M.C. Bibby & A.M. Matthew (introduced by M.E.Kelly), Clinical Oncology Unit, University of Bradford, Bradford BD7 1DP

A series of anti-cancer agents has been synthesised by Dr.R.S.McElhinney (Trinity College, Dublin, Eire) which combine the anti-metabolite 5-fluorouracil (5-FU) with the alkylating species N-(2-chloroethyl)-N-nitrosourea (CNU). Many are highly active against a panel of murine adenocarcinomas of the colon (MAC tumours); a good model for human colorectal cancer. 5-FU should be gradually released hydrolytically *in vivo* thus allowing attack by two drugs with different mechanisms of action. B.3970 (Figure 1) is an inactive isomer of the active B.3958 (Figure 1). The pharmacokinetics and pre-clinical toxicity of B.3958 have been studied previously (in preparation). This study describes preliminary pharmacokinetic data for B.3970 and the release profile of 5-FU in this model. Comparison of the isomers will be valuable in directing future chemical synthesis.

B.3970 was administered to NMRI mice at a dose of 100mg kg^{-1} (ip) in 10% DMSO/arachis oil. B.3970 and 5-FU were measured by HPLC (in preparation; Loadman *et al.*,1992).

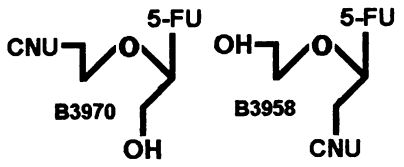

Figure 1.

Compound	Dose	C_{max}	T_{max}	K_{el}	$t_{1/2}$	AUC $_{0-\infty}$
	mg kg^{-1}	μg ml^{-1}	min	h^{-1}	min	μg h ml^{-1}
B.3970	100	29.2±9.6	33.8±16.3	0.82±0.22	53.7±13.2	38.2±15.3
Met 1	-	3.90±1.0	41.3±19.5	1.04±0.29	43.9±14.4	5.42±1.36
5-FU	-	0.69±0.13	120±0	1.69±0.18	25.7±2.90	1.12±0.23

Table 1. Summary of pharmacokinetic data for B.3970 (n = 4; ± 1 s.d.)

Combined results from two independent experiments are shown in Table 1. Pharmacokinetic calculations used standard formulae reported in Loadman *et al.*(1992). A metabolite (Met 1), more polar than B.3970 was detected and quantified as B.3970 equivalents (assuming the same extinction coefficient and peak area as B.3970). It has not yet been identified and it's cytotoxic properties are currently being investigated. Average peak levels (C_{max}) of B.3970 (29.2 μg ml^{-1}) and AUC values (38.2μg h ml^{-1}) are larger than for B.3958. Although 5-FU was detectable both peak plasma levels and AUCs are thought to be too low to be effective. The MTT assay (Jabbar *et al.*,1989), measuring percent cell survival after exposure to drug suggested that B.3970 is at least as cytotoxic as B.3958 against three of the MAC tumour lines grown as monolayers *in vitro* with IC_{50} values (μg ml^{-1}±1 s.d.) averaging 9.5±4.9 for B.3970 compared to 73.9±30.0 for B.3958. Plasma pharmacokinetic and drug metabolism data alone are not sufficient to explain the lack of anti-tumour activity of B.3970. Tumour levels must be measured to assess differences in drug penetration.

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170P CORRELATIONS BETWEEN FUNCTIONAL (ABSORPTIVE) DAMAGE AND STRUCTURAL (MORPHOMETRIC) CHANGES IN RAT SMALL INTESTINE CAUSED BY 5-FLUOROURACIL

M.L. Gardner, D.L. Steele & D. Wood, Department of Biomedical Sciences, University of Bradford, Bradford, West Yorkshire, BD7 1DP

Glucose and water absorption in perfused rat small intestine have previously been used to evaluate gastrointestinal toxicity of 5-fluorouracil (Gardner *et al.*, 1978). We have now explored the correlations between fluid absorptive activity and various morphometric indices of structural integrity throughout the time-course of intestinal damage after a single administration of 5-fluorouracil (5-FU).

Adult male Sprague-Dawley rats received a single injection of 5-FU (300mg/kg i.p.). At various times subsequently (1-42 days), the small intestines were removed under ether anaesthesia and luminally perfused with Krebs-Henseleit bicarbonate solution at 37°C containing 28mM glucose. Perfusate was recirculated by a gas-lift (95% O_2/5% CO_2). The serosally-transported fluid was collected every 10 min and weighed as an index of fluid absorptive activity. Each intestine was perfused for 65 min, and the length and dry weight then measured. Samples of unperfused proximal jejunum and distal ileum were taken for histological morphometry. Comparisons were made throughout with age-matched untreated rats since vehicle injection had no effect on the measured indices.

Administration of 5-FU caused severe intestinal damage, with an immediate and progressively dramatic decrease over the first 3 days in: body weight (to 89 ± 1% of control); jejunal villus height (from 600 ± 10 to 380 ± 20μm); jejunal crypt depth (230 ± 10 to 180 ± 20μm); small intestinal length (103 ± 1 to 92 ± 3cm); intestinal dry weight (15.2 ± 0.3 to 7.0 ± 0.2mg/cm); and total fluid absorption rate (16.6 ± 0.6 to 0.4 ± 0.2ml/h). (All values are means ± s.e. mean for 3-9 intestines; $P<0.05$ for all comparisons). Histologically, a loss of cellular organisation together with increased infiltration by inflammatory cells were observed. There followed a progressive recovery in all indices, so that by day 14 all values were not significantly different from the corresponding age-matched controls. Data from the whole-time course showed highly significant correlations ($P<0.005$) between water absorption rate and villus height (in jejunum or ileum) or crypt depth (in jejunum or ileum) or small intestinal length. Preliminary experiments (data not shown) gave similar results for mice injected with 5-FU or with a nitrosourea compound, thus suggesting that these results are not specific for the rat nor for 5-FU.

Not only do these results confirm that measurement of water absorption *in vitro* is a useful index of intestinal toxicity of 5-FU, but they also show that this functional index correlates very closely with indices of structural damage such as villus height and crypt depth. Further, much larger changes are seen in fluid absorption than in structural measurements which suggests that this functional measurement is a more sensitive index of damage. Hence, measurement of water absorptive activity is a particularly valuable procedure for inclusion in programmes to assess gastrointestinal toxicity of potential chemotherapeutic agents.

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171P ALDEHYDE OXIDASE CATALYSES FORMATION OF 5-HYDROXYINDOLEACETIC ACID IN GUINEA-PIG LIVER AND BRAIN

C.F. Peet, J.A. Smith & C. Beedham (introduced by C.H.K. Cheng), Pharmaceutical Chemistry, School of Pharmacy, University of Bradford, BD7 1DP

Oxidation of 5-hydroxyindoleacetaldehyde (5-HIAld) to 5-hydroxyindoleacetic acid (5-HIAA) is attributed to aldehyde dehydrogenase (ALDH) (Nilsson & Tottmar, 1987). The present study investigates the role of two other oxidising enzymes, aldehyde oxidase (AO) and xanthine oxidase (XO) (Beedham 1988) in 5-HIAld and thus 5-hydroxytryptamine (5-HT) metabolism.

5-HT (1mM) was incubated with precision-cut guinea pig liver slices, an integrated enzyme system containing MAO, ALDH, XO and AO in oxygenated Krebs-Heinseleit buffer pH 7.4 at 37°C. Disulfiram (0.1mM), allopurinol (1mM) and isovanillin (1mM) were added as specific inhibitors of ALDH, XO and AO respectively (Figure 1A). Incubations were also performed with frozen liver slices (Figure 1B) and brain homogenates. 5-HT and its metabolites were analysed by reversed-phase HPLC coupled to UV or EC detection.

In 5-HT incubations with fresh liver slices, 5-HIAA was the major metabolite. Up to 5% of 5-hydroxytryptophol (5-HTOL) was also found except in disulfiram incubations when up to 40% was formed in 45 min. In frozen liver slices, which retained AO but only contained 42±14% ALDH activity of fresh slices, 5-HIAA formation over 45 min was only decreased by 16±4% (mean ± s.e. mean).

When 5-HT (30μM) was incubated with guinea pig cortex or mid-brain homogenates 5-HIAA was also the major metabolite. Isovanillin (0.1mM) inhibited 5-HIAA production by 82±3% (n = 15).

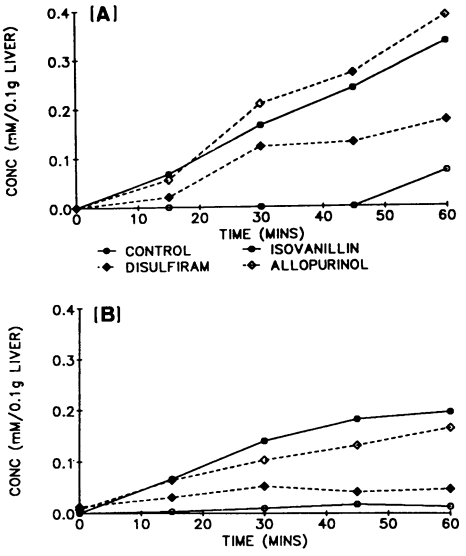

Figure 1. Formation of 5-HIAA in (A) fresh and (B) frozen guinea pig liver slices (n = 5)

These results show that 5-HIAld is a substrate for AO and that AO is the major enzyme responsible for 5-HIAA formation in guinea pig liver with a lower contribution from ALDH. XO does not appear to be important in this reaction. In addition, these preliminary experiments demonstrate brain AO activity for the first time and indicate its importance in 5-HT metabolism.

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172P AN INVESTIGATION OF DIDEOXYCYTIDINE NEUROTOXICITY IN THE RAT

L. Bleazard[1], R. Morris[1] and W.G. McLean, Department of Pharmacology and Therapeutics, and [1]Department of Veterinary Preclinical Sciences, University of Liverpool, PO Box 147, Liverpool L69 3BX

The dideoxynucleoside dideoxycytidine (ddC) is now in clinical use for the treatment of human immunodeficiency virus (HIV)-mediated acquired immune deficiency syndrome (AIDS), either independently or in combination with the more commonly used dideoxynucleoside 3'-azido-3-deoxythymidine (AZT). However the use of ddC is associated with a peripheral neuropathy which may turn out to limit the chronic use of the agent (Yarchoan *et al.*, 1988). In order to study the mechanism of this neurotoxicity, ddC (a gift from Roche Products, Ltd.) was administered to female Wistar albino rats by gastric intubation twice daily at two doses, 2mg/kg and 40 mg/kg for up to 19 weeks and three independent parameters of nerve function were measured. Control rats were treated with drug vehicle.

Motor nerve conduction velocity (MNCV) was measured on the exposed sciatic nerve of anaesthetized rats, by photography of oscilloscope recordings of compound action potentials. MNCV was 56.9 ± 3.3 m/sec (mean ± s.e. mean; n=8) in control rats and 55.4 ± 2.8 (n=9) in rats treated with 2mg/kg ddC for 8 weeks. Landing foot spread was equally unaffected by up to 40mg/kg ddC for 8 weeks (control: 92 ± 1mm (n=8); ddC: 92 ± 1mm (n=6))

Accumulation of immunoreactivity to the retrogradely transported protein synaptophysin was analyzed in pieces of sciatic nerve distal to a ligature applied unilaterally on the sciatic nerve under surgical anaesthesia. Synaptophysin immunoreactivity was measured by dot-blotting of nerve homogenates on to nitrocellulose membrane and incubation with a commercial anti-synaptophysin antibody followed by ^{125}I-radiolabelled secondary antibody and gamma-counting of bound radioactivity. Retrograde accumulation of synaptophysin immunoreactivity was unaffected by administration of 2mg/kg ODC for up to 19 weeks: ratios of immunoreactivity in nerve pieces distal to a crush over that in contralateral nerve pieces were 1.52 ± 0.12 in control rats (n=8) and 1.54 ± 0.13 in ddC-treated rats (n=8).

Induction of the enzyme ornithine decarboxylase (ODC) in dorsal root ganglia, which is known to be inhibited by neurotoxic agents (Myall *et al.*, 1990) was measured in rats in which one sciatic nerve had been crushed under surgical anaesthesia 24h previously. After treatment of rats with 40mg/kg ddC for 8 weeks the ratio of ODC activity in ganglia from crushed nerves over that from uncrushed contralateral nerves was 1.00 ± 0.08 (n=12), compared with a ratio in control rats of 1.69 ± 0.33 (n=12). This difference was statistically significant ($P<0.02$; Mann-Whitney 'U' test). We conclude that, despite the lack of overt neurological dysfunction in the rat, ddC can impair ODC induction, part of the regenerative process of damaged peripheral nerve. This would be of relevance in AIDS, in which peripheral neuropathy is a complication of the disease process itself (Cornblath *et al.*, 1987).

Supported by the Medical Research Council.

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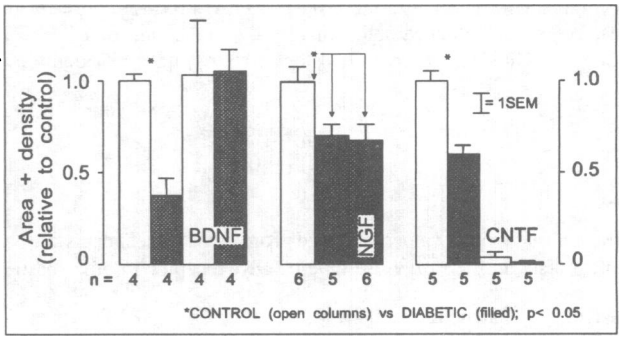
173P NERVE GROWTH FACTOR mRNA IN DIABETIC RAT SCIATIC NERVE: EFFECTS OF NEUROTROPHIC FACTOR TREATMENT

P. Fernyhough, W.J. Brewster, L.T. Diemel & D.R. Tomlinson Department of Pharmacology, Queen Mary and Westfield College, Mile End Road, London E1 4NS, U.K.

Experimental diabetes in rats is associated with reduced levels of a range of axoplasmic proteins; this may contribute to the development of diabetic neuropathy and may be explained by a diabetes-induced impairment of neurotrophic support (Tomlinson, 1992). This study was designed to measure the levels of nerve growth factor (NGF) mRNA in sciatic nerve and to determine the effects of treatment with NGF itself or ciliary neurotrophic factor (CNTF) or brain-derived neurotrophic factor (BDNF). Male Wistar rats were made diabetic with streptozotocin (50mg/kg i.p.) and sciatic nerves removed at death 4, 6 or 12 weeks later. NGF mRNA levels were measured by Northern blot hybridisation, with internal standardisation via addition of a synthetic truncated 'sense' cRNA, which hybridised to the same cDNA probe. The internal standard was added at the start of the RNA extraction.

Diabetic rats were hyperglycaemic and showed weight loss. NGF mRNA levels were reduced significantly ($p<0.05$) at 4 weeks (41% of controls) and at 6 weeks (59 and 72% of controls in separate experiments, $p<0.05$), but showed a 2-fold increase after 12 weeks of diabetes ($p<0.05$). In the 12 week study the increase in NGF mRNA levels in diabetic nerve was prevented completely by intensive insulin treatment (mRNA levels were significantly lower than those of untreated diabetic rats - $p<0.05$ - and not different from controls). Treatment with BDNF (0.9 mg/rat s.c. three times per week during 4 weeks diabetes) prevented the decrease in NGF mRNA. Treatment with NGF (0.5 mg/rat s.c. three times per week for the final 3 weeks of 6 weeks diabetes) was without effect on the reduced levels of NGF mRNA. Treatment with CNTF (1.0 mg/rat s.c. three times per week for the final 3 weeks of 6 weeks diabetes) abolished measurable traces of NGF mRNA. Levels of NGF mRNA (in densitometric units from image analysis of autoradiographs) in the three intervention studies are shown in the figure. These findings indicate that altered expression of NGF mRNA occurs in the sciatic nerve of rats with experimental diabetes and that the phenomenon can be modulated in either direction using exogenous neurotrophic factors.



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174P AMINOGUANIDINE ATTENUATES ENDOTHELIUM-DEPENDENT RELAXATION OF PRECONTRACTED AORTIC RINGS IN CONTROL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

M. Dewhurst, E.J. Stevens & D.R. Tomlinson *Department of Pharmacology, Queen Mary & Westfield College, Mile End, London*

Angiotensin II induced pressor responses of isolated rat lungs are potentiated in the presence of aminoguanidine (AG), which may be due to inhibition of endothelium-dependent relaxation (Eaton et al, 1992). This study therefore examined the effects of AG on acetylcholine (ACh) induced relaxations of rat aortic rings (in the presence of indomethacin to block cyclo-oxygenase products) as an index of nitric oxide release.

Three groups of male Wistar rats were used, the first (321 ± 3 g; aged 9 weeks) were made diabetic by injection of streptozotocin (50 mg/kg i.p.) and used 5 weeks later, the second were age matched and third weight matched to the first. Thoracic aortae were removed, cut into 3-4 mm rings, and suspended in organ baths (Krebs'-Henseleit, 37°C, pH 7.4, 95%O_2, 5%CO_2 with indomethacin $5x10^{-6}$M) for the recording of isometric tension. Cumulative concentration-response curves to phenylephrine were obtained for each group and an approximate EC_{65} used to induce tone (weight matched controls $3x10^{-7}$M, diabetic and age matched $1x10^{-6}$M). Concentration-relaxation responses were then obtained using ACh ($3x10^{-10}$-$1x10^{-4}$M) prior to and after incubation in either Krebs' fluid alone, N^G-nitro-L-arginine methyl ester (L-NAME; $1x10^{-5}$M) or AG ($1x10^{-5}$-$1x10^{-3}$M). ACh induced relaxation was blocked completely by L-NAME (10^{-5} M). Responses to phenylephrine were not significantly different for tissues from diabetic and weight matched animals however maximal responses (mean ± sem) were reduced in age-matched controls (9.59 ± 2.13 N; n=6) compared to diabetic (15.08 ± 1.54 N; n=6) and weight matched controls (13.85 ± 1.18 N; n=8). Likewise there were no differences in the degree of ACh-induced relaxation in aortae from weight matched control and diabetic rats, but significantly ($p<0.05$) greater relaxations were seen in rings from age matched controls. AG attenuated ACh-induced relaxations (mean ± SD) in a concentration-dependent fashion; this effect was significant ($p<0.01$ except where stated) at AG ($1x10^{-3}$M) and at ACh concentrations of $1x10^{-7}$M and $1x10^{-5}$M (respectively) for diabetic aortae (32 ± 16 to 10 ± 7 %) and (84 ± 10 to 59 ± 11 %; n=7-8), weight matched control aortae (47 ±13 to 21 ± 4 %) and (84 ± 6 to 69 ± 11 %; n=6-8; $p<0.05$) and age matched control aortae (73 ± 30 to 33 ± 22 %) and (99 ± 16 to 80 ± 14 %; n=8). Log EC_{50} values for acetylcholine were unchanged in all tissues, except for age matched controls, which were significantly ($p<0.01$) increased in the presence of AG (1mM).

These data indicate that aminoguanidine is capable of antagonising endothelium-dependent relaxations of rat aortic ring preparations obtained from control and diabetic tissues, although it is clearly less effective than other inhibitors such as L-NAME under the same experimental conditions.

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175P CHARACTERIZATION OF NEUROKININ RECEPTORS ON NG 108-15 CELLS

S. Maudsley, E. Carpenter & J.P. Gent, Department of Pharmacology, University of Leeds, Leeds LS2 9JT.

We have previously shown (Phenna *et al.*, 1992) that the NG 108-15 mouse/rat neuroblastoma x glioma cell line contains neurokinin receptors of the NK_1 type, as judged by responses to selective agonists. These receptors mediate reduction of voltage-gated Ca^{2+}-activated K^+ currents. Recently, the use of novel non-peptide NK_1 antagonists has indicated heterogeneity of NK_1 receptors (Regoli, 1992); it is proposed that they fall into two groups, guinea-pig/human type and rat/mouse type. We have now used the selective antagonists CP-99,994 (McLean *et al.*, 1992), GR82334 (Ireland *et al.*, 1992) and RP 67580 (Garret *et al.*, 1991) in order to characterize the NG108-15 receptor in more detail.

Whole-cell patch-clamp recordings were made from undifferentiated cells. The patch electrodes contained (in mM): KCl 117, EGTA 1, HEPES 11, $CaCl_2$ 1, $MgSO_4$ 2, NaCl 10, ATP 2 (pH 7.2), and the bathing solution was composed of (in mM): NaCl 135, KCl 5, $MgSO_4$ 1.2, $CaCl_2$ 2.5, HEPES 10, glucose 10 (pH 7.4, 21-24°C). The selective NK_1 agonist GR73632 (10^{-4}M in the latter solution) was applied by microiontophoresis from multibarrelled micropipettes with current compensation. The cells were clamped at a holding potential of -70mV. Depolarising steps of 100ms duration to +60 mV (0.1Hz) were used to evoke a prolonged outward potassium current. Five second ejections of GR73632 (100-1000 nA) produced dose-related responses consisting of a reproducible reduction in this current. Such responses were blocked competitively by application of CP-99,994, GR82334 and RP 67580 in the bathing medium. The dose-response curves to GR73632 were shifted to the right, and the Gaddum equation was used to calculate pK_B values (see Table 1).

Table 1. pK_B values in NG 108-15 cells (mean ± s.e. mean)

GR82334	CP-99,994	RP 67580
6.21 ± 0.17	7.15 ± 0.14	8.81 ± 0.07
n = 5	n = 6	n = 7

The estimated pK_B values obtained from these experiments on NG 108-15 cells are all in accord with the published pK_B values for these antagonists on the rat/mouse type of NK_1 receptor in other tissues and species.

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176P TISSUE-SPECIFIC CHANGES IN THE EXPRESSION OF LIPOCORTIN 1 (LC1) DURING POSTNATAL DEVELOPMENT IN THE RAT

R. Kikkert, H.D. Loxley, A.D. Taylor, R.J. Flower[1], C.Rantle, J.G. Philip & J.C. Buckingham. Dept. of Pharmacol., Charing Cross & Westminster Medical School, London W6 8RF and Dept. Biochem Pharmacol.[1], The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, London EC1M 6BQ.

In the neonatal rat the hypothalamo-pituitary-adrenocortical (HPA) axis is poorly developed and the adrenocortical response to stress is severely compromised. This "stress-hyporesponsive period" (SHRP) has been attributed in part to a supersensitivity of the steroid-sensitive components of the axis (i.e. hippocampus, hypothalamus, anterior pituitary gland) to the negative feedback actions of the glucocorticoids. Our previous studies have shown that in the adult rat lipocortin 1(LC1) is an important mediator of glucocorticoid feedback (Taylor et al., 1992; Loxley et al., 1993) but its role in the aetiology of the SHRP has not been examined. In the present study we have monitored the expression of LC1 in various brain and peripheral tissues throughout the SHRP and compared it to that in the adult. LC1 was determined by Western blotting and densitometry (Smith et al., 1993) in protein extracts of tissues [homogenized in 200mM phenylmethylsulfonyl fluoride (PMSF), 100mM NaCl, 5mM EDTA, 10mM HEPES and centrifuged (1,400g, 15min, at 4°C)]collected post mortem from male CFY rats aged 0, 5 10, 15, 30, and 42 (adult) days. Quantitative comparisons (37kDa band) were made only within blots and between lanes in which equal quantities of protein had been applied. Results are expressed as arbitrary units±SEM (n=5-7) and were analysed using the Mann-Whitney U-test. LC1 was readily detectable in all tissues examined, the predominant molecular species being the native (37kDa) form. In the hypothalamus and a number of peripheral tissues (spleen, liver, kidney) the levels were highest at birth and declined rapidly thereafter to the normal adult range [hypothalamus: 518±23 (0 days) vs. 236±29 (15 days) $p<0.001$; 835± 28 (15 days) vs. 887±20 (adult) NS; spleen: 503±71 (0 days) vs. 585±38 (15 days) NS; 741±58 (15 days) vs. 286±25 (adult) $p<0.001$; liver: 824±59 (0 days) vs. 486±23 (15 days) $p<0.001$; 1327±197 (15 days) vs. 1226±124 (adult) NS; kidney: 522± 35 (0 days) vs. 288±16 (15 days) $p<0.001$; 550±27 (15 days) vs. 178±7 (adult) $p<0.001$]. However, in the hippocampus and pituitary gland the LC1 content increased progressively during the post-natal SHRP reaching a maximum at 15 days [hippocampus: 314±23 (0 days) vs. 615±27 (15 days) $p<0.001$; 377±18 (15 days) vs. 147±15 (adult) $p<0.001$; pituitary: 406±19 (0 days) vs. 569±29 (15 days) $p<0.01$; 841±57 (15 days) vs. 842±58 (adult) NS]; a similar profile was evident in the testis: [921±56(0 days) vs. 1203±58 (15 days) $p<0.01$; 1551±138 (15 days) vs.,713±49 (adult) $p<0.001$]. In contrast, the LC1 content of the thymus decreased sharply in the SHRP [276±17 (0 days) vs. 148±28 (15 days) $p<0.001$] and increased thereafter [160±9 (15 days) vs. 272±13 (adult) $p<0.001$]. No significant age-related changes in LC1 expression were apparent in the brain cortex or the adrenal gland. The results demonstrated distinct, tissue selective developmental patterns of LC1 expression, many of which do not correlate positively with the known changes in the pattern of circulating glucocorticoids. The temporal association between the expression of LC1 in the hippocampus and pituitary gland (although not the hypothalamus) and the SHRP raises the possibility that LC1 contributes to the apparent tissue-selective receptor mediated supersensitivity to glucocorticoids (Meany et al., 1985). The striking fall in the LC1 content of some tissues observed post-natally may reflect the dramatic fall in circulating glucocorticoids which occurs at term.

We are grateful to the Wellcome Trust and Dr. Saal van Zwanenbergstichting for financial support. R.J.F. is funded by an endowment from Lilly.
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177P MODULATION OF THE RELEASE OF PROLACTIN AND THYROTROPHIN BY DEXAMETHASONE AND LIPOCORTIN 1

A. D. Taylor, R. J. Flower[1] & J. C. Buckingham. Department of Pharmacology, Charing Cross & Westminster Medical School, London W6 8RF and [1]Department of Biochemical Pharmacology, The William Harvey Research Institute, The Medical School of St. Bartholomew's Hospital, London EC1M 6BQ

Glucocorticoids inhibit the secretion of a number of anterior pituitary hormones including corticotrophin (ACTH), growth hormone (GH), prolactin (PRL) and thyrotrophin (TSH). Our previous studies have shown that the steroid-induced inhibition of ACTH release is mediated in part by a second messenger protein, lipocortin 1 (LC1, Taylor *et al*, 1992a & b) but the role of LC1 in the control of the secretion of the other anterior pituitary hormones remains to be investigated. In the present study we have used an *in vitro* model (Taylor *et al*, 1992a) to examine the influence of dexamethasone (dex), a neutralizing monoclonal anti-LC1 antibody (LC1-Ab) and an N-terminal LC1 fragment (LC1$_{1-188}$) on the resting and the neurochemically-evoked release of immunoreactive (ir-) PRL and ir-TSH from rat anterior pituitary tissue. The secretagogues used were hypothalamic extracts (HE), vasoactive intestinal peptide (VIP) and a Ca^{++} channel opener (BAY K8644) with contact times of 30, 60 and 60 min respectively. Data (mean ± SEM, n=5-6) were analysed by Duncan's multiple range test. Hypothalamic extracts (0.05-0.40 HE/ml), VIP (1pM-10nM) and BAY K8644 (10nM-100μM) all produced concentration dependent increases in the release of ir-PRL and ir-TSH. Submaximal concentrations (0.1HE/ml, 10nM VIP, 10μM BAY K8644), which increased basal ir-PRL release from 2.5±0.4 to 17.6±3.3ng/ml, 6.1±0.9 to 10.4±0.7ng/ml and 1.5±0.4 to 4.9±0.5ng/ml respectively and basal ir-TSH release from 3.9±0.7 to 25±4.9ng/ml, 5.8±1.7 to 16.4±2.7ng/ml and 2.8±0.5 to 12.5±2.5ng/ml respectively, were used subsequently. Preincubation of the tissue with dexamethasone (0.1μM, 2.5h) did not affect the spontaneous release of either ir-PRL or ir-TSH but reduced significantly ($P<0.05$) the increases in peptide release evoked by 10nM VIP (for ir-PRL, VIP=14.5±4.1ng/ml, VIP+dex=5.3±0.5ng/ml ; for ir-TSH, VIP=9.5±1.1ng/ml, VIP+dex=5.6±1.3ng/ml) but not by BAY K8644. Inclusion of the LC1-Ab (mouse anti-LC1, Zymed, diluted 1:15000) in the medium completely overcame the inhibitory effects of dexamethasone on the VIP-induced release of ir-PRL and ir-TSH but a correspondingly diluted control Ab (anti spectrin alpha + beta, Sigma Chemicals Co. Ltd.) was without effect. Following a 30min contact time, the spontaneous release of ir-PRL and ir-TSH was unaffected by LC1$_{1-188}$ (10pg/ml-1ng/ml) but, when the contact time was increased to 60min, LC1$_{1-188}$ caused significant ($P<0.01$) increases in the release of both ir-PRL [basal= 6.1±1.0ng/ml ; LC1(10pg/ml) = 11.4±2.2ng/ml] and ir-TSH [basal = 8.8±1.2ng/ml ; LC1(100pg/ml)= 29.7±3.9ng/ml]. LC1$_{1-188}$ (10fg/ml-1ng/ml) also reduced significantly ($P<0.01$) the ir-PRL responses to HE and VIP but not to BAY K8644. By contrast, it failed to influence the release of ir-TSH evoked by either HE or BAY K8644 although at certain concentrations (0.1 & 10ng/ml) it attenuated the ir-TSH responses to VIP.
The results suggest that the inhibitory actions of dexamethasone on ir-PRL and ir-TSH secretion are secretagogue specific and that they may be mediated, at least in part, by lipocortin 1.

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178P EVALUATION OF THE BEHAVIOURAL EFFECTS OF A NOVEL THYROTROPHIN-RELEASING HORMONE (TRH)-LIKE PEPTIDE (pGlu-Glu-ProNH_2,EEP) IN THE RAT

M. M. Mabrouk and G. W. Bennett, Department of Physiology and Pharmacology, Queen's Medical Centre, Nottingham NG7 2UH, U.K.

A TRH-like peptide (EEP) which differs from TRH by substitution of glutamic acid for histidine at the second residue of the tripeptide, was recently characterized in rabbit prostate and human semen (Cockle et al., 1989 a, b), and is also present in limbic regions of rat brain (Cockle & Bennett, 1993, personal communication). In this study various behavioural effects of EEP following intraperitoneal (i.p.) administration were measured and compared with the established effects of the neuropeptide, TRH (Bennett et al., 1989).

TRH or EEP (0.5, 1, 2, 5 and 10 mg kg^{-1}) and saline controls were injected i.p. into male Wistar rats (170-250 g) and the locomotor activity (L.A.) was recorded every 15 min for 90 min using photo-cell activity meters. In separate studies rats were similarly treated with EEP (i.p.) either alone or in some cases after pretreatment (-30 min) with haloperidol (0.2 mg kg^{-1} i.p.) or saline, then placed in a sound-proofed, temperature controlled enclosure and the behaviour monitored using a video camera and recorder. Various stereotypic behaviours, including forepaw licking (FPL), sniffing, rearing, chewing and wet-dog shake (WDS) behaviours were measured for 45 min post injection.

The results showed that TRH increased LA in a dose-related manner as described previously (Bennett *et al.*, 1989). Total activity counts at 90 min were mean ± s.e. mean (n = 7 or 8), saline (174 ± 14), TRH (2 mg kg^{-1}, 203 ± 48; 3.5 mg kg^{-1}, 209 ± 26; 5 mg kg^{-1}, 371 ± 23; and 10 mg kg^{-1}, 441 ± 31) which were highly significant ($p < 0.001$ from saline, Student's unpaired t test) at 5 and 10 mg kg^{-1}. EEP also increased LA, but in contrast with TRH, this was not dose-dependent and the smallest test dose (0.5 mg kg^{-1}) produced a similar though variable activity at 90 min (245 ± 46) compared to that of the highest tested dose (10 mg kg^{-1}, 262 ± 15) both highly significant responses ($p < 0.01$) compared with saline (111 ± 12, n = 12), while intermediate doses (eg. 5 mg kg^{-1}) showed intermediate increased ($p < 0.01$) activity (204 ± 11). The response at the latter dose in separate animals (166 ± 34) was also significantly inhibited ($p < 0.01$) following pretreatment with the dopamine antagonist, haloperidol (0.2 mg kg^{-1}, 49 ± 16). The stereotypic behaviours (such as FPL) and WDS both similarly increased in a dose-independent manner which reached significance in some cases, while haloperidol pretreatment significantly ($p < 0.05$) inhibited the EEP induced FPL but not WDS.

These studies indicate that the TRH-like peptide, EEP, produces hyperactivity and increased stereotypic behaviour similar to that of TRH but at an apparent increased potency at low responses and lacking a dose relationship. Moreover, the EEP behaviours, like the TRH-induced effects, may be mediated by central dopaminergic neurones and provide evidence that EEP may either interact with TRH receptors or possibly receptor sites separate from the TRH receptor, analogous to the effects shown by these two peptides on pituitary cells (Ashworth et al., 1993).

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179P EFFECT OF (+)-MK-801 ON THE INCREASE IN EXTRACELLULAR [K+] SEEN IN RAT DORSAL HIPPOCAMPUS, *IN VIVO*, DURING TRANSIENT HYPOXIA AS MEASURED BY ION-SELECTIVE MICROELECTRODES

M. D. R. Croning, D. G. Grahame-Smith & T. S. C. Zetterström, Oxford University SmithKline Beecham Centre for Applied Neuropsychobiology, University Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE.

The involvement of N-methyl-D-aspartate (NMDA) receptors in neuronal damage following ischaemia, hypoxia or anoxia has been demonstrated, *in vivo*, by several laboratories (see Benveniste, 1991). In support of this, *in vivo* microdialysis studies have shown that extracellular glutamate concentration increases sharply in the hippocampus during ischaemia (see Benveniste, 1991). Concomittant with the increased glutamate release, there is also evidence for the loss of cellular ionic homeostasis. For example, extracellular K^+ concentration ($[K^+]_e$) has been demonstrated to increase rapidly after the onset of hypoxia while the decrease in extracellular Ca^{2+} concentration occurs a little later (Hansen, 1982). The latter effect has been shown to be attenuated by NMDA receptor antagonists (see Benveniste, 1991), however, the mechanism behind the preceding $[K^+]_e$ increase is less certain. The possible involvement of NMDA receptors in mediating the early rise in $[K^+]_e$ in rat hippocampus, *in vivo*, was investigated in this study by the use of (+)-MK-801, a non-competitive NMDA receptor antagonist.

$[K^+]_e$ was measured using ion-selective, double-barreled microelectrodes based on the K^+ ionophore, valinomycin. The reference barrel of these electrodes was back-filled with 150 mM NaCl and the ion-selective barrel with 150 mM KCl. Electrodes were calibrated in saline-based solutions (constant ionic strength, K^+ 3-30 mM). Mean electrode response was 57.5 ± 2.77 mV/$\log_{10}$ concentration of K^+, n=3. Male Sprague-Dawley rats (250-300g) were anaesthetised with chloral hydrate (500 mg/kg i.p.) and mounted in a stereotaxic frame. Breathing gas mixture was regulated by anaesthetic apparatus (flow rate 1 l/min) and administered through a polyethene tube into which the rat's snout was placed. Body temperature was maintained at 37 ± 0.5 °C by heating blanket and rectal temperature probe. Microelectrodes were implanted in dorsal hippocampus. Transient hypoxia was induced by switching from the control gas mixture of 20% O_2 and 80% N_2 to 100% N_2 for 20 secs. Experimental protocol consisted of 3 stable control periods of hypoxia at 10 min intervals, followed by 3 test periods of hypoxia 30 minutes after drug administration (i.p.). Statistical analysis was made by the unpaired Student's t-test, comparing $[K^+]_e$ increases during control and test hypoxic periods; data presented as mean±s.e.m. or range.

Hypoxia induced an almost immediate increase in $[K^+]_e$ (0.517 ± 0.041 mM, n=14 rats), that was rapidly reversed following return to control gas mixture. (+)-MK-801 (1 mg/kg) significantly attenuated ($p<0.05$) this increase in $[K^+]_e$ by 50.1 ± 2.9%, n=4. In comparison, rats treated with vehicle (saline, 1ml/kg) showed a slight but insignificant ($p=0.548$) increase in $[K^+]_e$ (6.38 ± 5.9%, n=5). (+)-MK-801 at a dose of 3 mg/kg had a greater inhibitory effect (range: 62.8-85.6%, n=2) but it was lethal to 4/6 rats (inducing severe hypothermia), therefore it was not further tested. MK-801 at dose of 0.1 mg/kg i.p. had no significant effect ($p=0.968$) on $[K^+]_e$ rise during hypoxia (range: –9.15-3.74%, n=3).

In conclusion, these experiments demonstrate that at least part of the increase in $[K^+]_e$ seen during the early stages of hypoxia in the rat dorsal hippocampus may be dependent upon activation of NMDA receptors.

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180P QUANTITATIVE AUTORADIOGRAPHIC DISTRIBUTIONS OF GLUTAMATERGIC LIGAND BINDING SITES IN GOLDFISH BRAIN

J.M. Barnes and J.M. Henley, Department of Pharmacology, The Medical School, University of Birmingham, Birmingham, B15 2TT, United Kingdom.

A previous qualitative study of the distribution of [^{3}H]kainate binding in goldfish brain (Ziegra et al., 1990) indicated relatively high levels of binding in the cerebellum. The aim of the present study was to quantitate the distribution of glutamatergic ligand binding sites present in goldfish brain utilising the radioligands; [^{3}H]kainate, [^{3}H]AMPA ([^{3}H]alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate), [^{3}H]CNQX ([^{3}H]6-cyano-7-nitroquinoxaline-2,3-dione) and [^{3}H]L-glutamate.

In order to determine the distributions of radioligand binding, whole goldfish brains were homogenised individually in Tris-citrate buffer (50 mM; pH 7.4) and centrifuged (15400xg, 10 min, 4°C). The pellet was frozen (-80°C) and subsequently embedded in OCT and sectioned for autoradiography exactly as for intact brain sections.

The frozen sections of tissue (homogenate or intact brain; 20 µm) were thaw-mounted onto gelatine-coated glass slides. The slide mounted sections were pre-incubated in Tris-citrate buffer (37°C, 30 min) before incubation with radioligand, (60 min, 4°C) ([^{3}H]kainate (5 nM), [^{3}H]AMPA (10 nM) in the presence of 0.1M potassium thiocyanate, [^{3}H]CNQX (50 nM) or [^{3}H]L-glutamate (100 nM)) in the absence (total binding) or presence of competing drug (non-specific binding, defined by kainate (100 µM), AMPA (100 µM), CNQX (100 µM) and L-glutamate (1.0 mM), respectively). After washing, the sections were dried and exposed to [^{3}H]-Hyperfilm with [^{3}H]standards (Amersham) and, after developing, quantitated by image analysis.

Table 1. Specific binding of [^{3}H]kainate, [^{3}H]AMPA, [^{3}H]CNQX and [^{3}H]L-glutamate recognition sites in intact goldfish brain regions. Data represents mean±SEM, n = 3.

	Bound (fmol/mg grey matter tissue equivalent)			
	Telencephalon	Tectum	Cerebellum	Lobus Vagi
[^{3}H]kainate	498 ± 30	341 ± 34	1087 ± 100	394 ± 10
[^{3}H]AMPA	2114 ± 40	1594 ± 49	1046 ± 23	1803 ± 55
[^{3}H]CNQX	360 ± 23	6057 ± 387	3684 ± 286	338 ± 23
[^{3}H]L-glutamate	600 ± 7	243 ± 13	203 ± 14	340 ± 21

Each of the radioligands showed distinct regional distributions with binding sites for [^{3}H]L-glutamate being the most widely distributed. [^{3}H]kainate and [^{3}H]CNQX binding displayed widespread and superimposable distributions. The highest density of [^{3}H]kainate sites was detected in the cerebellum where it has been shown previously that [^{3}H]kainate binding is restricted to the molecular layer (Ziegra et al., 1990). It has yet to be established, however, whether this is at the non-neuronal class of sites located exclusively on Bergmann glia for which no function has yet been elucidated (Somogyi et al., 1990).

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181P KAINATE-EVOKED RELEASE OF PURINES FROM RAT HIPPOCAMPUS *IN VIVO*

H.V. Ogilvy and T.W.Stone, Department of Pharmacology, University of Glasgow, Glasgow. G12 8QQ, Scotland.

In hypoxia, ischaemia and other degenerative conditions excitatory amino acids are released into the extracellular space and are thought to mediate cell death by activating the N-methyl-D-aspartate, kainate and AMPA sub-types of glutamate post-synaptic receptors. Since purines have been shown to protect against kainic acid neurotoxicity (MacGregor and Stone, 1993) the present study investigates the release of adenosine induced by kainic acid in an *in vivo* microdialysis study of the hippocampus.

Wistar male rats 270-310g were anaesthetised with 1.25 g kg^{-1} i.p. urethane. Concentric dialysis probes, 0.3 mm in diameter, made with 4 mm Hospal polyacrylonitrile membrane were stereotaxically inserted into the hippocampus (5.6 mm posterior, 5.0 mm lateral, 8.0 mm ventral relative to the bregma). Artificial CSF (ACSF) was pumped at 2 μl min^{-1} through the probes, samples being collected every 20 min and analysed for purine content by HPLC. A 5 min pulse of kainic acid 0.1 to 5 mM was introduced into the ACSF 2 h 40 min after probe insertion, once basal levels of the purines were achieved.

The table shows the effect on release (pmols 20 min^{-1}) of purines at four times following a 5 min pulse of 1 mM kainic acid.

	Collection time (min) after kainate infusion				
	0 (basal)	20	40	60	80
Adenosine	14.52±1.29	20.05±2.16*	39.09±7.01*	25.86±3.60*	18.29±1.85*
Inosine	13.99±1.09	17.35±0.71*	30.98±4.2*	37.73±3.65*	30.61±4.24*
Hypoxanthine	25.38±2.65	34.68±3.06*	106.84±16.59*	155.32±15.29*	127.87±23.00*
Xanthine	75.28±8.03	85.23±9.66*	87.92±7.30	121.67±9.08*	137.27±9.10*

All values are mean ± s.e. mean for n=10. *$P<0.05$, using a paired t-test.

The kainate-evoked release could be blocked by the non-NMDA receptor antagonist CNQX 4.5 μM (6-cyano-7-nitroquinoxaline-2,3-dione), indicating a non-NMDA receptor-mediated release.

We therefore conclude that kainic acid is able to evoke an increase in the extracellular concentration of endogenous adenosine and its metabolites. This correlates with *in vitro* studies by Hoehn & White (1990) and may explain the ability of adenosine antagonists to potentiate kainate neurotoxicity (MacGregor & Stone, 1993).

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182P DOSE-DEPENDENT STIMULATORY EFFECTS OF SOME ANAESTHETIC AGENTS ON ISOLATED MOUSE VAS DEFERENS

M R Hirji and P V Taberner, Department of Pharmacology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, England

Many anaesthetics, including the dissociative anaesthetics phencyclidine (PCP) and ketamine, have been shown to inhibit the electrically-induced contraction of smooth muscle (Mayer et al., 1981). In some cases this inhibition can be reversed by naloxone, suggesting that opiate receptors are involved in the inhibitory action (Blum et al.,1980, Gintzler, 1982). However, PCP and ketamine also possess the ability to block noradrenaline (NA) re-uptake into noradrenergic neurones (Taube et al., 1975). Since the electrically evoked contractions of the mouse vas deferens are due to release of NA from sympathetic nerve terminals we have examined the dose response relationship of a range of dissociative and other anaesthetics on this preparation to determine whether any sympathomimetic action can be detected.

Vasa from adult LACG mice were set up singly under 250 mg tension in a 5 ml bath at 37°C in Mg^{++}-free Krebs Ringer bicarbonate medium (pH 7.4) gassed with 95% O_2: 5% CO_2. Isometric responses were recorded following electrical stimulation (60-100V, 0.1 Hz, 0.5 msec duration). After 1h equilibration to establish a consistent baseline response, drugs were added in a cumulative fashion with a 30 min washout between drugs. ED_{50} values were derived from dose-response curves fitted to the logistic equation. Results are means ± s.e.mean from 3 to 6 mice.

At bath concentrations between 1 and 80 µM the following drugs all produced dose-dependent increases in the size of electrically evoked contractions (ED_{50} values, µM): ethanol, 27 ± 3.0; etomidate, 34 ± 2.0; alphaxalone, 12 ± 1.2; trichloroethanol, 18 ± 2.5; PCP 6.0 ± 1.0; and ketamine, 7.0 ± 1.5. The maximum potentiation ranged from 80% (ethanol and ketamine) to 200% (alphaxalone) of the control value. Over the same dose ranges none of these drugs altered the response of unstimulated tissue to the addition of standard doses of NA (1 - 20 µM). At concentrations in excess of 100µM all these drugs produced a dose-dependent inhibition of the electrically-induced contractions which was reversible on wash-out.

The results suggest that sub-anaesthetic concentrations of a number of anaesthetics can act pre-synaptically to increase the effects of sympathetic nerve stimulation. There are several means by which this effect could be brought about and further experiments, using tyramine and cocaine and tissues preloaded with [^{3}H]-NA, are intended to determine whether a common mechanism of action exists.

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183P INTERACTION OF RAUWOLSCINE AND DESIPRAMINE ON NORADRENALINE EFFLUX IN RAT BRAIN SLICES

P. Palij & J.A. Stamford. Anaesthetics Unit, London Hospital Medical College, Whitechapel, London E1 1BB.

Antidepressant drugs do not generally exert any clinical effect until after 2-3 weeks of chronic administration. The lag between onset of pharmacological and clinical effects suggests counteracting mechanisms are at work, delaying the appearance of clinical benefit. Desipramine (DMI), a tricyclic antidepressant, has been shown to selectively inhibit noradrenaline (NA) reuptake. Previously (Palij & Stamford, 1992) we observed that DMI had a smaller effect on NA efflux than uptake. In this study we investigated the possibility that this might be due to enhanced autoreceptor activation, in turn accounting for the discrepancy between the onset of uptake blockade and the clinical antidepressant effect.

Fast cyclic voltammetry at carbon fibre microelectrodes was used to monitor stimulated NA efflux in slices of the rat bed nucleus of stria terminalis as previously described (Palij & Stamford, 1992). Efflux was evoked using electrical stimulation (trains of 30 pulses, 0.2ms, 10mA, applied at 100Hz every 5 min) at bipolar stimulating electrodes. Drugs were added after the sixth train (S_6). The responses to S_{25}-S_{27}, expressed as a percentage of the mean of the responses to S_1-S_6, were compared to time-matched controls and differences analysed by one-way analysis of variance followed by Duncan's multiple range test.

Drug(s)	Control	Rauwolscine	DMI	Rauwolscine + DMI
NA efflux (Δ%)	-9.0 ± 8.3	+11.8 ± 6.9	+60.0 ± 24.6*♦	+127.5 ± 14.0 *♦♦
NA uptake $t_{½}$ (Δ%)	+1.1 ± 7.5	-3.8 ± 5.3	+262.1 ± 62.3*♦	+378.4 ± 60.4*♦

*Percentage change relative to S_{1-6} (means ± s.e.m, n=4). *$P < 0.05$ vs controls, ♦$P < 0.05$ vs rauwolscine, ♦$P < 0.05$ vs DMI.*

The α_2 antagonist rauwolscine (1μM) had no effect upon NA efflux or uptake half-life ($t_{½}$). DMI (50nM), alone and with rauwolscine, significantly ($P<0.05$) increased NA efflux and prolonged $t_{½}$. The facilitation of efflux produced by the DMI/rauwolscine combination was significantly greater ($P<0.05$) than that produced by DMI alone although the prolongation of $t_{½}$ was not significantly different.

The potentiation of DMI by rauwolscine suggests that, following DMI, autoreceptors are activated and counteract the effects of uptake blockade on NA efflux. Rauwolscine blocks this autoinhibition and thus reveals the full effect of DMI on NA efflux. A similar action of DMI *in vivo* may underlie the lack of acute antidepressant effect: enhancement of synaptic NA levels by blockade of uptake may increase activation of autoreceptors and thereby inhibit further release. Conversely, after chronic DMI, autoreceptor down-regulation would be expected to diminish the autoinhibition of NA release thereby unmasking the therapeutic effect of DMI.

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184P HAEMOLYTIC PROPERTIES OF PLURONIC SURFACTANTS: EFFECTS OF PURIFICATION

K.C. Lowe, B.A. Furmidge & S. Thomas, Dept. Life Science, University of Nottingham, Nottingham NG7 2RD

Pluronic F-68 is a non-ionic surfactant present in the O_2-carrying perfluorochemical emulsion, *Fluosol*® (Alpha Therapeutic), being used as an adjunct to coronary balloon angioplasty (Lowe, 1992). A Pluronic F-68 formulation, *RheothRx*® (Burroughs-Wellcome), is also being studied for improving blood rheology (Raymond, 1989). A pre-requisite to such uses is assessment of Pluronic effects on blood cells. The effects have therefore been studied of incubating mammalian blood with Pluronic F-68 or Pluronic F-38 and their fractions *in vitro*. Solutions (10% w/v) of commercial grade (COMM) Pluronic F-68 and Pluronic F-38 (BASF Corporation) were prepared in isotonic saline (0.9% w/v NaCl) and purified by passing 3 times through a glass column containing silica resin (Bentley *et al.*, 1989). Removal of contaminants was confirmed by measurement of UV absorption (200-400 nm) following each passage through the column. Blood was obtained from rats, mice, hamsters or rabbits and placed in plastic tubes containing heparin (Sarstedt). 50 μl aliquots of blood were added to 5 ml of 0.025-10.0% (w/v) of either COMM or purified (PUR) Pluronic F-68 or Pluronic F-38 in 0.9% (w/v) NaCl. Samples were incubated in a water bath at 37°C for 60 min., centrifuged at 3000 rpm for 5 min, and supernatants aspirated. Haemolysis was measured spectrophotometrically (Al-Assadi *et al.*, 1990).

COMM Pluronic F-68 showed an absorption band with peaks at 210-215 nm (absorbance 2.7 A.U.) and 270-280 nm (absorbance 0.4 A.U.), as seen previously (Bentley *et al.*, 1989). COMM Pluronic F-38 had a similar absorption band with peaks at 205-210 nm (absorbance 2.4 A.U.) and 240-290 nm (absorbance 0.2 A.U.). After passage through the silica column, the higher UV absorption band for both compounds was undetectable (< 0.005 A.U.). Incubation of blood with up to 4% (w/v) of either COMM or PUR Pluronic F-68 produced no significant haemolysis, but haemolysis did occur with COMM Pluronic F-68 concentrations above 4% (w/v), with a species-dependent response. Incubation of rat blood with 10% (w/v) COMM Pluronic F-68 produced 4.7 ± 1.5 % (n = 3) haemolysis; this was significantly ($P < 0.05$) reduced when 10% (w/v) PUR Pluronic F-68 was used (0.5 ± 0.3 %). Incubation of rat or hamster blood with COMM or PUR Pluronic F-38 produced no significant haemolysis (< 0.1%), irrespective of concentration. Incubation of rabbit blood with COMM Pluronic F-38 produced a maximum of 0.5% haemolysis at 10% (w/v); this was only marginally lower with the PUR fraction. While caution is needed in extrapolating from *in vitro* to *in vivo* systems, these results suggest that PUR Pluronic F-68 may be preferable in formulations for intravascular applications. This supports previous findings (Bentley *et al.*, 1989) that purification of Pluronic F-68 reduces liver weight increases in rats injected with this compound. The species variability in red cell responses to Pluronic F-68 was consistent with earlier work using poly(oxyethylene) ether surfactants (Al-Assadi *et al.*, 1990) and may reflect differences in erythrocyte membrane composition. The finding that neither COMM nor PUR Pluronic F-38 had significant haemolytic activity is difficult to explain given that its poly(oxypropylene) content is identical to that of Pluronic F-68 and that both compounds have similar permeabilizing effects on yeast (Laouar *et al.*, 1992). One explanation is differences in haemolytically-active impurities between COMM Pluronic F-68 and COMM Pluronic F-38, but this needs clarification.

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185P COMPUTER-SIMULATED ARTERIAL AND VENOUS RING PREPARATIONS FOR TEACHING VASCULAR PHARMACOLOGY

I.E. Hughes & M.S. Yates. Department of Pharmacology, University of Leeds, Leeds LS2 9JT, Yorkshire, UK.

Several computer programmes are available which simulate in vivo or in vitro pharmacological preparations (Hughes, 1987;1989;1991). Such programmes have a role in the teaching of pharmacology, particularly to students for whom training in pharmacological laboratory skills is of lesser importance. Furthermore, even with students for whom good laboratory skills are essential, simulations can be used successfully to broaden experience, to provide a fall-back when experiments fail, to better prepare for real laboratory classes, and to teach experimental design. In the latter cases simulation of vascular responses is particularly useful, since in the laboratory, individual responses may take minutes to develop, leaving little room for error in experimental design within the time constraints of a laboratory practical session.

This computer programme (IBM or compat; VGA) simulates the responses of arterial rings with either endothelium present or denuded of endothelium and an arterial ring removed from an animal previously treated with *E. coli* endotoxin. In addition, the responses of a venous ring with endothelium intact are also simulated. Output is in the form of a "trace" either on screen or as hard-copy on a standard printer. The drugs available have been chosen to allow a range of experiments to be performed to illustrate and investigate vascular pharmacology. The drugs include noradrenaline, potassium, isoprenaline, phenylephrine, tyramine, 5-hydroxytryptamine, substance P, endothelin-1, clonidine, UK-14,304, nitroprusside, acetylcholine, angiotensin, $PGF_{2\alpha}$, BAY K 8644, N^G-monomethyl-L-arginine, prazosin, atropine, hexamethonium, idazoxan, phentolamine, rauwolscine, 17-β-oestradiol, phenoxybenzamine, desmethylimipramine, propranolol, ketanserin, atenolol, methysergide, cocaine and nifedipine. The simulation is very flexible since the concentration of drug employed and the sequence of drug administration are determined by the user. Teachers can design experiments suitable for their own students or can use all or part of the background information, instructions and work schedules provided in machine readable form on the disc. Alternatively, teachers can ask the student to design and carry out experiments to answer a set question. The programme will also provide "unknown agonists" the properties and potency of which can be determined by the student.

While this simulation is not designed to replace entirely laboratory practicals in this topic, it does provide an instructive alternative which may prove very useful in the teaching of both science and medical students.

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186P A COMPUTER SIMULATION OF THE FINKLEMAN PREPARATION TO TEACH UNDERGRADUATE PHARMACOLOGY STUDENTS

D.G. Dewhurst, I.E. Hughes[1] and R.T. Ulyott, Faculty of Health & Social Care, Leeds Metropolitan University, Calverley Street, Leeds LS13HE, and [1]Department of Pharmacology, University of Leeds, Leeds LS2 9JT.

In recent years a number of computer simulations of pharmacological preparations designed to augment, or in some instances with appropriate students, to replace conventional teaching methods have been demonstrated to the Society (e.g. Dewhurst et al, 1992a; 1992b; Hughes, 1989;1991). Here we describe a simulation of the isolated duodenal preparation of the rabbit, with sympathetic nerves attached (Finkleman preparation) designed to teach the pharmacology of the sympathetically-innervated intestine *in vitro*.

The program is written in C++ (Borland Turbo C++) for IBM-compatible microcomputers running a range of graphics drivers (EGA, VGA). **Introduction and Methods** sections combine text and high-resolution graphics to describe the preparation, the apparatus and to provide the background pharmacology. In the **Experiments** section simulated rhythmic contractions, derived from experimental data, are presented on a chart-recorder-like display on the monitor. The user is able to select, from a menu, to investigate:

(1) a range of adrenergic drugs, either alone or in certain pre-determined combinations, (noradrenaline, isoprenaline, phenylephrine, tyramine, guanethidine, imipramine, alpha- and beta-adrenoceptor blockers);

(2) the effects of electrically stimulating sympathetic nerves (supramaximal stimulus voltage; 1-50 Hz); or

(3) the effects of a range of drugs (guanethidine, imipramine, lignocaine, tetrodotoxin, alpha- and beta-adrenoceptor blockers) on the response to sympathetic nerve stimulation (supramaximal stimulus voltage, 50 Hz).

The drugs, drug combinations and electrical stimulation protocols have been selected to demonstrate the essential pharmacology of the preparation. The program is accompanied by printed support materials and may be used in association with a teacher-designed protocol or for independent learning.

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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 **Zetterström TSC & Grahame-Smith DG** The measurement of cerebral extracellular K^+ by ion-selective microelectrodes *in vivo*: effect of hypoxia and modulation by K^+ channel blocking drugs

2P **Rose S, Jenner P & Green AR** Chlormethiazole does not protect against MPP^+-induced nigral toxicity in rats

3P **Dexter DT, Nanayakkara I, Goss-Sampson MA, Muller DPR, Marsden CD & Jenner P** The effect of chronic vitamin E deficiency on striatal dopamine levels, reduced glutathione and tyrosine hydroxylase positive cells in rat substantia nigra

4P **Large C, Stubbs CM, Mills A, Kawashima E & Hayes AG** A comparison of the benzamide pharmacology of rat and human dopamine D_3 receptors expressed in Chinese hamster ovary cells

5P **Farrell CB & O'Boyle KM** Partial conversion of D_1 dopamine receptors from high to low agonist affinity by Gpp(NH)p, heat and N-ethylmaleimide

6P **Bull DR, Hayes AG & Sheehan MJ** A clozapine-insensitive response to the dopamine agonist, N,N-dipropylamino-5,6-dihydroxytetralin (dpADTN): regional localisation and effect of pertussis toxin

7P **Britton P, Whitton PS, Fowler LJ & Bowery NG** Effects of tetanus toxin on basal and K^+-evoked extracellular concentrations of 5-HT, dopamine and their metabolites in rat hippocampus measured by *in vivo* microdialysis

8P **Cadogan AK, Kendall DA & Marsden CA** 5-HT_{1A} receptor-activation increases cAMP efflux in the rat hippocampus *in vivo*

9P **Skingle M, Scopes DIC, Feniuk W, Connor HE, Carter MC, Clitherow JW & Tyers MB** GR127935: a potent orally active 5-HT_{1D} receptor antagonist

10P **Brown AM, Young TJ, Patch TL, Cheung CW, Kaumann AJ, Gaster L & King FD** [^{125}I]-SB 207710, a potent, selective radioligand for 5-HT_4 receptors

11P **Del Rio J, Artaiz I, Zazpe A, Romero G, Monge A, Roca J & Lasheras B** VA-21B7: An atypical 5-hydroxytryptaminergic anxiolytic

12P **Rodgers RJ, Cole JC & Tredwell JM** Ethological analysis fails to reveal anxiolytic effects of 5-HT_3 receptor antagonists, ondansetron and WAY 100,289, in the murine plus-maze

13P **Shepherd JK, Grewal SS, Fletcher A, Bill D & Dourish CT** Pharmacological evaluation of the elevated "zero-maze" as a model of anxiety in rats

14P **Boden P & Woodruff GN** Effects of benzodiazepines on CCK_B responses recorded from rat ventromedial hypothalamus *in vitro*

15P **Wardle KA, Ellis ES, Gaster LM, King FD & Sanger GJ** SB 204070: a highly potent and selective 5-HT_4 receptor antagonist

16P **Bingham S, King BF, Rushant B, Smith MI & Sanger GJ** The 5-HT_4 receptor antagonist SB 204070 inhibits the contractile response to 5-HT in the dog Heidenhain pouch

17P **Banner SE, Smith MI, Bywater D & Sanger GJ** 5-HT_4 receptor antagonism by SB 204070 inhibits 5-hydroxytryptophan-evoked defaecation in mice

18P **Chamienia AL & Johns EJ** The haemodynamic and renal functional responses to the 5-HT_{1A} receptor agonist flesinoxan in the anaesthetised rat

19P **Munavvar AS & Johns EJ** The role of α_1-adrenoceptor subtypes in the regulation of renal tubular processes in genetic hypertension

20P **Chess-Williams R, Jackson AH, Fabri BM & Fox MA** An investigation of the receptors mediating the responses of the human saphenous vein to phenylephrine

21P **Wright IK, Kendall DA & Wilson VG** α_2 adrenoceptor-mediated inhibition of forskolin-stimulated cAMP accumulation in the porcine palmar lateral vein

22P **Kelly E & Williams RJ** Chronic ethanol increases basal, agonist- and forskolin-stimulated cyclic AMP accumulation in NG108-15 cells

23P **Lawson K, Barras M, Armstrong JM & Hicks PE** Effects of glibenclamide and charybdotoxin on relaxations to the potassium channel activator BRL-55834 in guinea-pig trachea

24P **Wyatt CN & Peers C** Actions of doxapram on Ca^{2+}-activated K^+ channels from isolated type I carotid body cells of the neonatal rat

25P **Olesen S-P, Wätjen F & Hayes AG** A novel benzimidazolone, NS004, activates large-conductance Ca^{2+}-dependent K^+ channels in aortic smooth muscle

26P **Dunn PM** Dequalinium, a selective blocker of the K^+ channel underlying the slow after-hyperpolarization in rat sympathetic neurones in culture

27P **Diemel LT, Brewster WJ, Fernyhough P & Tomlinson DR** The effect of administration of nerve growth factor on calcitonin gene-related peptide (CGRP) expression in sciatic primary afferents of diabetic rats

28P **Stevens EJ & Tomlinson DR** Nerve ischaemia in diabetic rats: time-course of development and effects of insulin or aldose reductase inhibition

29P **Gardiner SM, Rakhit T, Kemp PA, March JE & Bennett T** Regional haemodynamic responses to pituitary adenylate cyclase-activating peptide and vasoactive intestinal polypeptide in conscious rats: effects of N^G-nitro-L-arginine methyl ester (L-NAME)

30P **Lot TY & Wilson VG** Haemoglobin causes endothelium-dependent contractions of the porcine isolated splenic artery that are sensitive to cyclooxygenase and lipoxygenase inhibitors

31P **Szabó Cs, Wu CC, Thiemermann C & Vane JR** Role of the constitutive and inducible nitric oxide synthases in the cardiovascular response in systemic anaphylaxis in the rat

32P **Smith JA, Evans M & Evans RA** Low density lipoproteins acutely inhibit EDRF activity in rabbit aorta by a mechanism involving protein kinase C but not superoxide anions

33P **Gelzer A & Ball HA** Cardiovascular effects of an inhibitor of nitric oxide formation in the anaesthetised minipig: pressure-volume analysis

34P **Goldsmith PC, Hayes NA, Dowd PM & Foreman JC** Inhibitory effect of N^G-nitro-L-arginine methyl ester (L-NAME) on vasodilation in human skin induced by intradermal calcitonin gene-related peptide (CGRP)

35P **Benito Lobo I & Hoult JRS** Groups I, II and III extracellular phospholipases A_2: selective inhibition of group II enzymes by indomethacin but not other NSAIDs

36P **de las Heras B, Villar A, Vivas JM & Hoult JRS** Comparison of *in vitro* properties of two novel anti-inflammatory plant labdanes with aspirin, sodium salicylate and indomethacin

37P **Carroll MA, Balazy M & Macica C** Regional distribution of cytochrome P450-arachidonate (P450-AA) metabolites in rabbit intestine

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39P **Milne AAY & Piper PJ** An antibody to interleukin-5 inhibits antigen-induced airway hyperresponsiveness and cell accumulation in guinea-pigs

40P **Charalambous K & Hoult JRS** Functional stimulation and calcium overload toxicity in peritoneal leukocytes elicited in rats by glycogen or interleukin-1β

41P **Poole AW, Blake R, Asselin J & Watson SP** Tyrosine kinase inhibitors block the increase in Ca^{2+} that occurs on the adhesion of single platelets to collagen

42P **Humphries RG, Tomlinson W, Ingall AH, Kindon ND & Leff P** FPL 66096: a novel, highly potent and selective antagonist at human platelet P_{2T}-purinoceptors

43P **Williams RJ & Kelly E** Biphasic desensitization of prostacyclin receptor responsiveness in NG108-15 cells

44P **Kengatharan M, Brown TJ, Raeburn D & Roach AG** Study of endothelin receptor subtypes mediating contraction of rat aorta and trachea using agonists and putative antagonists

45P **Bacon CR, O'Reilly G, Cameron IT & Davenport AP** Endothelin receptor subtypes in human myometrium characterized by BQ123 and BQ3020

46P **Battistini B, Allcock GH, Warner TD & Vane JR** ET_A and ET_B receptors mediate the release of eicosanoids from the rat kidney induced by endothelin/sarafotoxin peptides

47P **Maguire JJ & Davenport AP** Endothelin-induced vasoconstriction in human isolated vasculature is mediated predominantly via activation of ET_A receptors

48P **Macarthur H, Wood EG, Nair S, Henderson B, Warner TD & Vane JR** Stretching, both acutely and chronically, of endothelial cells increases their production of prostacyclin and endothelin

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50P **Desai KM, Warner TD, Bishop AE, Polak JM & Vane JR** *In vitro* and immunohistochemical studies suggest that nitric oxide, and not VIP, mediates vagally-induced gastric relaxation in the guinea-pig

51P **Baxendale AJ & Spraggs CF** The stimulatory and inhibitory effects of sCCK-8 in rat isolated gastric mucosa are mediated through different CCK receptors

52P **Hughes SJ & Hollingsworth M** Evidence that relaxin acts predominantly at the level of the plasmalemma in the isolated rat uterus

53P **Yard N & Ball HA** Effect of cyclopiazonic acid, an inhibitor of sarcoplasmic Ca^{2+}-ATPase, on the force-frequence relation of guinea-pig isolated atria

54P **Dimech J, Feniuk W & Humphrey PPA** Somatostatin receptors mediating negative inotropy in guinea-pig isolated right atrium

55P **Jolkkonen J, Jenner P & Marsden CD** Effect of GABAergic stimulation on basal striatal peptide expression and that induced by dopamine antagonist treatment

56P **Guard S, Horwell DC, Howson W, Hughes J, Pritchard MC, Roberts E, Rees D, Watling KJ & Woodruff GN** Rational design of high affinity NK_1 and NK_2 tachykinin receptor ligands

57P **Pradier L, Ménager J, Le Guern J, Bock MD, Heuillet E, Fardin V, Doble A & Mayaux JF** Septide: an agonist of the NK_1 receptor acting at a site distinct from substance P

58P **Barr AJ & Watson SP** Inhibitory feedback regulation of NK_1 tachykinin receptor signalling by protein kinase C in human UC11 astrocytoma cells

59P **Grimson P, Guard S, Woodruff GN & Watling KJ** [^{125}I]-[MePhe^7]Neurokinin B may label species variants of the NK_3 binding site in guinea-pig and rat cerebral cortex

60P **Lambert PD, Wilding JPH & Bloom SR** Interactions between neuropeptide Y and the κ-opioid system in the central control of food intake

61P **Petterson EKE, Hunter JC, Poat JA & Hughes J** The effect of enadoline on immediate early gene expression in the rat paw formalin test

62P **Hayward NJ, Hitchcott PK, McKnight AT & Woodruff GN** Effect of enadoline on ischaemia-induced glutamate release

63P **Jones RSG & Dhillon A** Phenytoin reduces the frequency-dependent facilitation of glutamate-mediated synaptic transmission in the rat entorhinal cortex

64P **Ripley TL & Little HJ** Concurrent chronic treatment with a dihydropyridine calcium channel antagonist prevents the effect of chronic ethanol treatment on tetanus-induced long term potentiation

65P **Dennes RP, Clapham J, Kilpatrick GJ & Barnes JC** Ability of the selective histamine H_3 receptor antagonist thioperamide to improve short-term memory and reversal learning in the rat

66P **Young KW & Young JM** Potentiation by DTT of histamine-induced [^{3}H]-IP accumulation in rat cerebral cortex

67P **Al-Qatari M & Taberner PV** Effect of aldehyde dehydrogenase inhibitors on brown adipose tissue ethanol metabolism and lipogenesis in CBA mice

68P **Roe S, Cooper AL, Morris ID & Rothwell NJ** Mechanisms of cachexia induced by T-cell leukaemia in the rat

69P **Peers C, Rana B, McMorn SO, Reeve HL, Wyatt CN & Vaughan PFT** Inhibition of neuronal nicotinic acetylcholine receptors in the human neuroblastoma SH-SY5Y by desipramine and imipramine

70P **Koenig JA & Edwardson JM** Delivery of muscarinic ACh receptors to the plasma membrane in unstimulated and agonist-stimulated NG108-15 cells

71P **Jenkinson S, Challiss RAJ & Nahorski SR** Disruption of phosphoinositide signalling by lithium in CHO-cells expressing recombinant muscarinic M_1-receptors

72P **Watson N & Eglen RM** Investigation of the role of muscarinic M_2 receptors in modulating relaxant responses to isoprenaline in guinea-pig isolated trachea

73P **Sudlow AW, Carey F & Rothwell N** Interactions between lipocortin-1 and the hypothalamic-pituitary-adrenal (HPA) axis

74P **Ford AJ & Longridge DJ** The effect of dexamethasone on lipopolysaccharide-induced disseminated intravascular coagulation in the anaesthetised rat

75P **Waller J, Gardiner SM & Bennett T** Differential effects of lipopolysaccharide on the carotid haemodynamic responses to acetylcholine and methoxamine in conscious Long-Evans rats

76P **Suitters AJ, Bodmer MW & Foulkes R** Antibody isotype affects the efficacy of anti-tumour necrosis factor antibodies on LPS-induced shock in mice

77P **Ward PS, Woodger SR, Bodmer M & Foulkes R** Anti-tumour necrosis factor α monoclonal antibodies (anti-TNF MAb) are therapeutically effective in a model of colonic inflammation

78P **O'Malley KE, Joyce P & Baird AW** Neuronal contribution to immediate hypersensitivity reactions in isolated colonic mucosa

79P **Keely SJ, Stack WA, O'Donoghue DP & Baird AW** Histamine stimulation of human colonic ion transport is mediated via H_1 receptors

80P **McCreath G, Hall IP & Hill SJ** Agonist-induced desensititation of the histamine H_1-receptor in human umbilical vein endothelial cells is independent of protein kinase C

POSTER COMMUNICATIONS

81P **Kidd EJ, Michel AD & Humphrey PPA** Regional autoradiographic localisation of nitric oxide synthase in the rat brain using [^{3}H]nitroarginine

82P **Aldridge CR & Collard KJ** High-affinity uptake of L-arginine by cerebellar and cortical synaptosomes

83P **Tucker LM, Emson PC & Morton AJ** Behavioural recovery following intrastriatal lesions with quinolinic acid is not related to survival of nitric oxide synthase-positive neurones

84P **Laszlo F, Boughton-Smith NK, Whittle BJR & Moncada S** Potentiation and inhibition of endotoxin-induced vascular injury in rat intestine by nitric oxide synthase inhibitors

85P **Buckley C, Bund S, McTaggart F, Bruckdorfer KR, Jacobs M & Oldham A** Oxidised low-density lipoproteins inhibit endothelium-dependent relaxations of rabbit coronary arteries

86P **Parsons S, Garland CJ & Plane F** The role of nitric oxide in acetylcholine-evoked dilatation of the rat isolated mesenteric bed

87P **Mickley EJ, Warner TJ & Vane JR** L-canavanine inhibits the recycling of L-citrulline to L-arginine in the rat anococcygeus muscle

88P **Khawaja AM, Perez AC, Paul W, Page CP & Schachter M** Effect of D- and L-arginine on contractile responses to histamine and bradykinin in guinea-pig ileum *in vitro*

89P **Dhiman MK, Lang D, Smith JA & Lewis MJ** Guanethidine inhibits endothelium-dependent relaxation in rabbit aorta

90P **Cutler MG** Behavioural effects of apomorphine in female mice

91P **Thabit M & Goudie AJ** The dopamine uptake inhibitor GBR 12909 induces cross-tolerance to rate-suppressant actions of cocaine in rats

92P **Brown CD & Barnes NM** Angiotensin II stimulates dopamine release from rat striatal slices via the AT_1 receptor

93P **Bowen WP, Coldwell MC, Hicks FR, Riley GJ & Fears R** Ropinirole, a novel dopaminergic agent for the treatment of Parkinson's disease with selectivity for cloned dopamine D_3 receptors

94P **Alam AM & Starr MS** D_1 agonists suppress epileptiform activity in the rat cortical slice

95P **Halford JCG & Blundell JE** 5-Hydroxytryptaminergic drugs compared on the behavioural sequence associated with satiety

96P **Costall B, Kelly ME & Naylor RJ** A potential involvement of the 5-HT_4 receptor in behavioural responding to an aversive situation?

97P **Ashworth-Preece MA, Hartley JE & Fletcher A** Involvement of 5-HT_{1A} receptors in morphine antinociception in rats and mice: a possible species difference

98P **Ebenezer IS** Effects of gepirone and buspirone on operant food intake in non-deprived rats

99P **Rudd JA, Bunce KT & Naylor RJ** The effect of 5-HT_{1A} receptor ligands on copper sulphate-induced emesis in the ferret

100P **Smith AG, Domeney AM, Costall B, Naylor RJ & Tyers MB** Alosetron attenuates rebound hyperactivity induced by withdrawal from a chronic mesolimbic dopamine infusion and systemic neuroleptic treatment

101P **Costall B, Kelly ME & Naylor RJ** Interaction between 5-hydroxytryptophan and 5-hydroxytryptamine receptor antagonists to inhibit and disinhibit rat social interaction

102P **Wood MD, Glen A, Blackburn TP, Lee JA, Sutiphong JA, Carey J & Robinson J** (–)-Fluoxetine has high affinity for the cloned rat and human 5-HT_{1C} receptor and the human 5-HT_2 receptor

103P **Harder JA, Kelly ME, Cheng CHK & Costall B** Combined para-chlorophenylalanine and muscarinic antagonist treatment produces a deficit in water maze acquisition

104P **Watson CD, Fone KCF, Jackson HC & Bennett GW** Effect of the stable TRH analogue RX77368 (pGlu-His-3,3'-dimethylProNH_2) on two cognitive tests in the senescent rat

105P **Hogg S, Costall B, Kelly ME, McCurrie JR & Salako A** Cognitive deficits following carotid occlusion in the rat in the absence of CA_1 cell death

106P **Mohiuddin L, Fernandes K, Tomlinson DR & Fernyhough P** Neurotrophic factors enhance regeneration and elevate growth-associated protein mRNAs in adult sensory neurones

107P **Rodgers RJ & Cole JC** Anxioselective effects of bretazenil (Ro16-6028) in the murine elevated plus-maze: comparison with chlordiazepoxide

108P **Watson WP & Little HJ** Diltiazem displays a biphasic effect on *in vivo* binding of [^{3}H]-nitrendipine

109P **Ripley TL & Little HJ** Effects of chronic treatment with ethanol, with and without a dihydropyridine calcium channel antagonist, on calcium-induced long term potentiation

110P **Green SJ & Wilson JF** Methadone is incorporated into hair by saturable processes

111P **Clayton RA, Templeton AGB & MacLean MR** α_2-adrenoceptor and 5-HT_1-mediated responses in bovine pulmonary arteries: the effect of vascular tone and forskolin

112P **MacLean MR, McCulloch KM, Templeton AGB & Baird M** Comparison of rabbit neonate and adult pulmonary arterial responses to 5-hydroxytryptamine

113P **Templeton AGB, Clayton RA, McIntyre PD, Hillis SW, Peacock AJ & MacLean MR** Sensitivity of human, bovine and rabbit pulmonary arteries to sumatriptan: comparison with 5-HT

114P **Gilani AH, Saeed SA & Suria A** Spasmolytic activity of himbadine in isolated guinea-pig ileum and rabbit jejunum

115P **Hemmings FJ, Grace RH & Birch NJ** Magnesium as an inhibitor of smooth muscle contraction in the human large bowel

116P **Beavington TE, Morrison JFB, Robertson AS & Neal DE** Muscarinic and β-adrenoceptor binding sites in the normal and unstable human bladder

117P **Kelso EJ, McDermott BJ & Silke B** Cardiotonic actions of the novel vasodilator, flosequinan, and its metabolite, BTS 53544, in isolated ventricular cardiomyocytes

118P **Moran RJ, Struthers AD & Hewick DS** Effect of digoxin-specific Fab fragments on renal function in the rat

119P **Dawson AC, Gwilt M & Wray D** Effect of chronically high insulin levels on potassium channels in rat ventricular myocytes

120P **Rees SA & Curtis MJ** Lack of effect of clofilium on repolarisation in the rat isolated heart confirms that rat ventricle is devoid of functional delayed rectifier potassium current

121P **Banning MA & Curtis MJ** Ischaemia-induced and reperfusion-induced arrhythmias in the isolated rat heart are suppressed by cimetidine without haemodynamic or electrocardiographic alteration

122P **Rouse W, Stafford PJ & Johnson IR** ZD7288, a novel selective sino-atrial node function modulator, compared with alinidine, zatebradine and β-adrenoceptor antagonists in exercising conscious rats

123P **Crinnion JN, Homer-Vanniasinkam S, Hatton R, Parkin SM & Gough MJ** The role of neutrophils in reperfusion injury

124P **van der Graaf PH, Welsh NJ, Shankley NP & Black JW** Evidence for heterogeneity of α_1-adrenoceptors in the rat aorta

125P **Sithers AJ, Marshall RW & Spriggs TLB** Reversible α_1-adrenoceptor agonist action of chloroethylclonidine in the rat anococcygeus muscle

126P **Davies AR, Marshall RW & Spriggs TLB** Tetraethylammonium enables activation of α_{1A}-adrenoceptors in the rat vas deferens stimulated by single electrical field pulses

127P **Shahid M, Chipperfield K & Nicholson CD** Tissue-dependent differences in the mechanism of cyclic nucleotide phosphodiesterase (PDE) IV isoenzyme inhibition by both rolipram and denbufylline

128P **Alderson G, Hambly RJ & Cross T** Can a streptomycete arthrosphore model be of value in detecting novel calcium-channel blocking agents?

129P **Taylor C & Baird AW** Berberine inhibits electrogenic chloride secretion in rat colon *in vitro*

130P **Davies MP, McCurrie JR & Wood D** Comparative effects of potassium channel openers on ^{86}rubidium efflux from rat ileum

131P **Ford AJ & Longridge DJ** The effect of a selective thrombin inhibitor on lipopolysaccharide-induced disseminated intravascular coagulation in the anaesthetised rat

132P **Burleigh DE & Borman RA** Characterisation of the 5-HT receptor mediating electrogenic fluid secretion in human small intestine

133P **Cheng CHK, Costall B, Mason K, Naylor RJ & Rudd JA** Effect of 5-HT_3/5-HT_4 receptor ligands on *in vitro* K^+-stimulated [^{3}H]5-HT release from rat cortical slices

134P **Leung E, Alvarez R, Bonhaus DW, Clark RD, Johnson LG, Lee C-H, Smith WL, Wong EHF, Whiting RL & Eglen RM** (R) and (S) 56532: mixed 5-HT_3 and 5-HT_4 receptor ligands with opposing enantiomeric selectivity

135P **Banner SE, Smith MI & Sanger GJ** 5-HT receptors and 5-hydroxytryptophan-evoked defaecation in mice

136P **Bufton KE, Steward LJ & Barnes NM** Assessment of 5-HT_3 receptor recognition site levels in the putamen of patients with Huntington's chorea and Parkinson's disease

137P **Martin GR, Browning C & Giles H** Further characterisation of an atypical 5-HT receptor mediating endothelium-dependent vasorelaxation

138P **Crick H & Wallis DI** Endogenous and exogenous 5-HT access different receptors to depress the monosynaptic reflex

139P **Sayer TJO, Martin KF & Redfern PH** Control of 5-HT by the terminal hypothalamic autoreceptor during the light:dark cycle

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141P **Barrett VJ, Prentice DJ, Martin JF & Martin GR** Comparison of adenosine receptors mediating negative inotropy in guinea-pig and human atrial preparations

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144P **Reeves JJ, Coates J, Jarvis JE & Sheehan MJ** Adenosine A_1 receptor-mediated contractions in rat colon are both inhibited and potentiated by 8-phenyltheophylline (8-PT)

145P **Carruthers AM & Fozard JR** Hypotensive responses to the putative adenosine A_3 receptor agonist, N^6-2-(4-aminophenyl)ethyl adenosine (APNEA) in the rat

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148P **Travers A, Robertson MJ, Clark KL & Drew GM** Is the renal papilla involved in the antihypertensive effect of the angiotensin AT_1 receptor antagonist, GR117289?

149P **Slee S-J & Wilson C** Inhibition of diatrizoate-induced reduction in renal blood flow by an endothelin antagonist

150P **Allcock GH, Warner TD & Vane JR** The endothelin receptor antagonists BQ-123 and PD 145065 reverse established vasoconstrictor responses to endothelin-1 *in vitro*

151P **Butler A, Beattie DT & Connor HE** Human α and β CGRP-mediated responses *in vitro*: sensitivity to the CGRP receptor antagonist, $CGRP_{8-37}$

152P **Kelley AS & Hollingsworth M** The P_1-purinoceptor of the guinea-pig taenia coli

153P **Woods M & Baird AW** Modulation of bradykinin stimulation of ion transport by guinea-pig gallbladder

154P **Abbas F, Marshall K & Senior J** The effects of bradykinin and related compounds on isolated human myometrium from pregnant and non-pregnant donors

155P **Pabla R & Curtis MJ** L-NAME-enhanced susceptibility to reperfusion-induced ventricular fibrillation is prevented by L-arginine in rat isolated heart

156P **Wu CC, Szabó Cs, Thiemermann C & Vane JR** Role of endogenous tumour necrosis factor, interleukin-1 and platelet-activating factor in the vascular hyporeactivity in rats treated with endotoxin

157P **Piotrowski W, Simon MC & Brennan L** Effects of N^w-Nitro-L-arginine and methylene blue on non-adrenergic, non-cholinergic responses of isolated guinea-pig taenia caeci

158P **Yanaga F & Watson SP** Role of sphingomyelin metabolism in superoxide generation by TNFα in human neutrophils

159P **Shaw GR, Keddie JR, Pinkney J, Clarkson PN, Growcott JW & Poucher SM** Vitamin E offers protection against the impairment of endothelium-mediated relaxation in the cholesterol fed rabbit aorta but not in the hind limb

160P **Krane A & Keen M** The effect of colchicine on IP prostanoid receptor desensitization in NG108-15 cells

161P **Kengatharan M, Brown TJ, Raeburn D & Roach AG** Age-related changes in potency of endothelin-1 and endothelin-3 occur in the rat aorta but not in the trachea

162P **Hicks R & Hemati AA** Selective enhancement of myofibroblast-activated contractility in paraquat pretreated rat lung tissue

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164P **Yeadon M, Jones HCW & Payne AN** Antigen-induced contraction of guinea-pig trachea and parenchyma: effect of the 5-lipoxygenase inhibitor BW B70C and modulation by indomethacin and mepyramine

165P **Malata CM, Naylor IL & Timmons M** Subcutaneous silicone implants in the rat: a model for capsular contracture?

166P **Li Q, Yates MS & Bowmer CJ** Effect of glycine on cisplatin-induced acute renal failure in the rat

167P **Michael-Titus AT, Basra S, Whelpton R & Grahn M** Tissue distribution in tumour-bearing mice of a new agent (5,10,15,20-tetra[m-hydroxyphenyl]chlorin) for photodynamic therapy of cancer

168P **Loadman PM, Hulbert PB & Bibby MC** Metabolism and pharmacokinetics of the nitrosourea B.3996

169P **Loadman PM, Bibby MC & Matthew AM** Plasma pharmacokinetic studies on B.3970, a molecular combination of 5-fluorouracil and N-(2-chloroethyl)-N-nitrosourea (CNU), in a murine model

170P **Gardner ML, Steele DL & Wood D** Correlations between functional (absorptive) damage and structural (morphometric) changes in rat small intestine caused by 5-fluorouracil

171P **Peet CF, Smith JA & Beedham C** Aldehyde oxidase catalyses formation of 5-hydroxyindoleacetic acid in guinea-pig liver and brain

172P **Bleazard L, Morris R & McLean WG** An investigation of dideoxycytidine neurotoxicity in the rat

173P **Fernyhough P, Brewster WJ, Diemel LT & Tomlinson DR** Nerve growth factor mRNA in diabetic rat sciatic nerve: effects of neurotrophic factor treatment

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