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303P CHARACTERISATION OF [^{3}H]-CI-977 LABELLED κ OPIOID BINDING SITES IN RODENT CNS

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The recent development of the kappa-selective arcylacetamides (Lahti et al, 1985; Clark et al, 1988) has allowed a detailed investigation of the characteristics and distribution of kappa opioid sites in the CNS. The most recent addition to this series of compounds is CI-977, a highly kappa-selective opioid agonist (Hunter et al, 1990). In the present study we have examined the characteristics of [^{3}H]-CI-977 binding and distribution in the forebrains of the rat and guinea-pig.

The methodology for ligand binding experiments and for autoradiography was as previously described (Clark et al, 1988, Smith et al, 1989). [^{3}H]-CI-977 bound with high affinity to an apparent single population of non-interacting sites in both rodent forebrain preparations. The equilibrium dissociation constant (K_D) obtained for [^{3}H]-CI-977 in the guinea-pig was 0.10 ± 0.1nM (90% specific binding) with a maximal binding capacity (Bmax) of 94.3 ± 5.8 fmol/mg protein. In the rat the K_D value obtained was 0.24 ± 0.02nM (70% specific binding) with a lower Bmax value of 19.3 ± 1.8 fmol/mg protein.

In competition studies against [^{3}H]-CI-977 (0.01nM) the high affinity of unlabelled CI-977, U69593 and PD117302 combined with the low affinity of the mu and delta selective ligands DAGOL and DPDPE were consistent with a kappa receptor profile (Table 1).

Table 1. K_i (nM) (Mean ± Standard Error for n experiments)

	CI-977	U69593	DAGOL	DPDPE	PD117302	BREMAZOCINE	NALOXONE
Guinea-Pig	0.19±0.02 (7)	1.65±0.44 (6)	280±53 (6)	>30000 (6)	1.03±0.20 (5)	0.14±0.04 (7)	5.00±1.00 (5)
Rat	0.17±0.02 (7)	4.39±1.20 (4)	307±73 (5)	>30000 (4)	1.43±0.09 (4)	0.09±0.01 (4)	4.48±1.19 (4)

The autoradiographic pattern of distribution was also consistent with [^{3}H]-CI-977 binding to kappa receptors in both rat and guinea-pig forebrain. Thus the highest levels of [^{3}H]-CI-977 binding in the guinea-pig were to be found in the interpeduncular nucleus (IPN), substantia nigra (SN) and cortical layers V and VI, whilst in the rat the highest levels were present in the IPN, SN and nucleus accumbens although the absolute levels were much lower than in the guinea-pig.

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304P CHARACTERIZATION OF THE PHARMACOLOGY OF SIGMA OPIOID SITES IN GUINEA-PIG BRAIN AND NCB20 CELL MEMBRANES

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Sigma binding sites, which have been postulated to mediate the psychotomimetic actions of benzomorphan drugs (Zukin *et al* 1981), have been characterised in the CNS (Weber *et al*, 1986). In order to determine whether NCB20 cells can be used as a model system to study mammalian sigma sites, the pharmacology of the sigma binding site in membranes prepared from these cells and guinea pig whole brain P_2 membranes has been compared, using the potent and specific ligand [^{3}H]-Di-tolyl-guanidine (DTG). Sigma binding was performed by incubating aliquots of membranes with 5nM [^{3}H]-DTG for 90 minutes at 23°C. Non specific binding was defined with 1μM haloperidol. Scatchard analysis of [^{3}H]-DTG binding in NCB20 cells revealed the site density to be 4.37 ± 0.13pmol/mg protein (n = 3) and the K_d 90.2nM (+ 98.5, - 81.9nM) (geometric mean, + sem, - sem). A range of sigma ligands were tested in both tissues (see Table). All of the compounds were 1.7 to 0.2 log units less potent in NCB 20 cell membranes. The (-) isomers of SKF 10,047 and pentazocine were more potent than the (+)-isomers in NCB20 cells whereas the reverse was true in guinea pig brain. (+) SKF 10,047, (+) pentazocine and (+)3PPP displaced sigma binding in guinea pig brain membranes with low Hill coefficients, suggesting heterogeneity in [^{3}H]-DTG binding sites. In NCB20 membranes no heterogeneity was apparent with any of the compounds tested.

Displacement of [^{3}H]-DTG (5nM) radioligand binding in guinea pig whole brain and NCB20 cell membranes

	G.P. brain		NCB20 Cells	
	pIC_{50}	nH	pIC_{50}	nH
DTG	7.49 ± 0.04	1.04 ± 0.03	6.93 ± 0.06	1.19 ± 0.07
(+)-SKF 10,047	5.69 ± 0.10	0.48 ± 0.05	4.13 ± 0.10	0.9 ± 0.08
(-)-SKF 10,047	5.0 ± 0.13	0.82 ± 0.09	4.42 ± 0.06	0.72 ± 0.07
(+)-Pentazocine	6.92 ± 0.18	0.49 ± 0.06	5.18 ± 0.33	1.08 ± 0.20
(-)-Pentazocine	6.84 ± 0.02	0.83 ± 0.07	6.65 ± 0.14	1.15 ± 0.13

Figures represent mean ± SEM, n = 3

Thus although haloperidol sensitive [^{3}H]-DTG binding is present in NCB20 cells, there are differences in the pharmacology seen in these membranes and in guinea pig brain, notably differing affinity, reverse stereoselectivity and Hill coefficients close to unity.

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305P SHORT-TERM LITHIUM INCREASES THE RELEASE OF HIPPOCAMPAL 5-HT EVOKED BY ELECTRICAL STIMULATION OF THE DORSAL RAPHÉ NUCLEUS IN THE RAT *IN VIVO*

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The addition of lithium to other antidepressant drugs is reported to have a rapid therapeutic effect in otherwise drug resistant depression (De Montigny et al, 1983). This rapid antidepressant effect of lithium might relate to an increased release of 5-HT in the brain which is proposed to occur in rats on the basis of earlier biochemical and behavioural measurements of presynaptic 5-HT function (Green & Grahame-Smith, 1974) and more recent electrophysiological studies of serotoninergic neurotransmission (Blier & De Montigny, 1985). Previously, we have shown using the microdialysis technique that electrical stimulation of the dorsal raphe nucleus (DRN) evokes a release of 5-HT in the rat hippocampus in vivo (Sharp et al, 1989). Here we have examined whether electrically-evoked release of hippocampal 5-HT is altered in rats receiving short-term treatment with lithium. Microdialysis was carried out as previously described (Sharp et al, 1989) in control or lithium chloride-treated (3 mmol/kg s.c. twice daily for 3 days), chloral hydrate-anaesthetized rats. Perfusates were collected every 20 min and analysed for 5-HT by HPLC-EC. Following a baseline period of up to 3 h, the DRN was electrically stimulated (300 μA,1.0 msec) for 20 min at 1 h intervals. The current frequency was increased successively with each period of stimulation (2, 3, 5 and 10 Hz). In control rats, electrical stimulation of the DRN evoked a frequency-dependent, short-lasting release of 5-HT in hippocampus. The effect of electrical stimulation was enhanced in rats treated with lithium particularly when low frequency stimulation was used (table 1). Basal 5-HT output was not significantly different between the lithium-treated and control groups.

Table 1 Effect of short-term lithium treatment on electrically-evoked release of hippocampal 5-HT in vivo

Frequency (Hz)	2	3	5	10
Control	0.016 ± 0.003 (9)	0.030 ± 0.009 (9)	0.045 ± 0.01 (8)	0.076 ± 0.025 (4)
Lithium	0.049 ± 0.004 (6)**	0.060 ± 0.003 (6)*	0.071 ± 0.005 (6)	0.106 ± 0.013 (4)

(values are absolute amounts of 5-HT (pmol) released during 20 min electrical stimulation at the pulse frequency indicated; mean ± S.E.M. (n), **p<0.001, *p<0.05 versus appropriate control according to Student's t-test)

In conclusion, short-term administration of lithium enhances electrically-evoked release of 5-HT in rat hippocampus in vivo. Increased availability of 5-HT in the brain could explain the therapeutic effect of lithium in drug-resistent depression.

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306P ELECTROPHYSIOLOGICAL STUDY ON THE EFFECTS OF DOI ON DORSAL RAPHÉ 5-HT NEURONAL FIRING

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DOI (2,5-dimethoxy-4-iodophenylisopropyl-amine) a relatively selective agonist at the 5-HT_2 receptor, with affinity for the 5-HT_{1C} receptor (McKenna *et al.*, 1989). DOI excites neurones in the medial prefrontal cortex at low application currents (0.5nA) but inhibits them at higher currents (20-80nA), and these effects are blocked by 5-HT_2 antagonists (Ashby *et al.*, 1989). Intravenous administration of DOI produces a decrease in dorsal raphe nucleus (DRN) neuronal firing (Garratt and Marsden, 1989), and reduces frontal cortex extracellular levels of 5-HT and 5-HIAA (Wright *et al.*, 1990). The aim of this study was to identify if the inhibition of DRN firing produced by DOI was due to activation of 5-HT_2 receptors situated on 5-HT neurones in the DRN.

Male Wistar rats were anaesthetised with halothane (1.5-2%) and the jugular vein was cannulated. Recordings were made with single barrelled glass electrodes in the DRN and 5-HT neurones were identified by their characteristic slow, regular firing pattern and inhibitory response to the 5-HT_{1A} agonist, 8-OHDPAT. Ketanserin (100μg/kg i.v.), a 5-HT_2 antagonist, or ritanserin (0.5mg/kg i.v.), a 5-HT_2/5-HT_{1C} antagonist, were administered 20 minutes prior to DOI (50μg/kg. i.v., a dose previously shown to decrease DRN firing by 60%). In microiontophoretic studies rats were anaesthetised with urethane (1.3g/k.g. i.p.), and a 5-barrelled micropipette was lowered into the DRN.

Administration of DOI (50μg/kg i.v.) markedly reduced firing (62 ± 6% of basal level); an effect not blocked by prior administration of either ketanserin or ritanserin. Microiontophoretic application of 8-OHDPAT or DOI inhibited DRN 5-HT neuronal firing in a dose related manner with complete inhibition of firing seen with an ejection current of +90nA. The sensitivity of raphe neurones to iontophoretic ejection (e.g. +60nA) of DOI (57.1 ± 7 % decrease of basal firing rate) was similar to that seen with 8-OHDPAT (61 ± 2.1% of basal firing rate).

These results suggest that DOI does not inhibit raphe neuronal firing via classical 5-HT_2 receptors as the effect was not antagonised by ketanserin. Furthermore the reduction of DRN firing appears to involve a different receptor from the effects previously observed in the cortex (Ashby *et al.*, 1989), and it is unlikely that this inhibition is mediated via the 5-HT_{1C} receptor as the response was not blocked by ritanserin. The iontophoretic studies indicate that the DOI mediated inhibition of DRN 5-HT neuronal firing is a direct effect on the 5-HT neurones. The receptor subtype at which DOI exerts this effect remains unclear and warrants further investigation.

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307P 5-HT_3 RECEPTOR AGONISTS ENHANCE THE ELECTRICALLY-EVOKED RELEASE OF [^{3}H]-5-HT IN GUINEA-PIG FRONTAL CORTEX SLICES

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5-HT_3 receptors present both in the periphery and in the CNS (Peroutka, 1988 ; Richardson et al, 1985) are associated with depolarization (Richardson and Engel, 1986), and electrophysiological results suggest that these receptors could be coupled to membrane ion channels (Derkach et al, 1989). It was reported that 5-HT_3 receptor agonists can inhibit acetylcholine release in cerebral cortex slices (Barnes et al, 1989), and we considered of interest to study the effects of 5-HT_3 receptor agonists on the release of [^{3}H]-5-HT from guinea-pig frontal cortex slices, preloaded with [^{3}H]-5-HT.

Two periods of electrical stimulation (3Hz, 2ms, 30mA, 2 min) were applied 98 min (S_1) and 142 min (S_2) after the onset of superfusion. Drugs were added 8 or 20 min before S_2 and 20 min before S_1. Exposure to the 5-HT_3 receptor agonist 2-methyl-5-HT(0.001 - 1 µM) 8 min before S_2 enhanced in a concentration-dependent manner the electrically-evoked release of [^{3}H]-5-HT (maximal effect at 0.1 µM : S_2/S_1 = 1.85 ± 0.15, n=8 $p<0.001$ when compared with the control value S_2/S_1 = 1.09 ± 0.06, n=7) without modifying the spontaneous outflow of radioactivity. When 2-methyl-5-HT (0.1 µM) was added to the medium 20 min before S_2, neither the [^{3}H]-5-HT overflow (S_2/S_1 = 0.98 ± 0.07, n=6, not different from the control value) nor the spontaneous outflow of radioactivity were modified. The 5-HT_3 receptor antagonists ICS 205930 (0.001 - 0.01 µM) and odanserin (0.01 - 0.1 µM) which by themselves did not modify the electrically-evoked release of [^{3}H]-5-HT, competitively antagonized the enhancing effect of 2-methyl-5-HT (S_2/S_1 = 0.93 ± 0.06, n=6 for 2-methyl-5-HT 0.1 µM in the presence of 0.01 µM ICS 205930 ; S_2/S_1 = 1.12 ± 0.06, n=7 for 2-methyl-5-HT 0.1 µM in the presence of 0.1 µM odanserin. The selective 5-HT_3 receptor agonist phenylbiguanide (0.01 µM) added to the superfusion medium 8 min before S_2, significantly enhanced the [^{3}H]-5-HT overflow (S_2/S_1 = 1.41 ± 0.09, n=6 $p<0.05$ when compared with the control value) and this effect was antagonized by 0.01 µM ICS 205930 (S_2/S_1 = 0.97 ± 0.10, n=7).

It is concluded that a presynaptic facilitatory 5-HT_3 receptor could modulate the electrically-evoked release of [^{3}H]-5-HT in slices of guinea-pig frontal cortex and that this receptor can undergo rapid desensitization following exposure to the agonist. The physiological significance of these results remains to be determined.

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308P EFFECT OF 5-HT ON REFLEX RESPONSES OF NEONATE RAT SPINAL MOTONEURONES

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Although 5-HT depolarizes rat motoneurones (Connell & Wallis, 1988) and increases their excitability, it depresses reflex responses evoked by dorsal root stimulation (Saito et al, 1982). We have investigated the actions of 5-HT on monosynaptic and polysynaptic reflex responses recorded from a ventral root evoked from the segmental dorsal root.

The lumbar spinal cord was removed from 3-8 day old rats anaesthetized with ether. The cord was hemisected and superfused with oxygenated modified Krebs solution. Stimulation and recording were via glass suction electrodes and the amplitudes of two components of the reflex response were monitored by a peak height detector with a double gate. Supramaximal square wave stimuli of 5-15V, 0.1 msec in duration, were applied at 0.07Hz.

Superfusion of the cord with different concentrations of 5-HT induced a concentration-related depression of both the monosynaptic and polysynaptic reflex. The IC_{50} for depression of the monosynaptic reflex was 9.5 ± 3.2uM (n=7), while the IC_{50} for depression of the polysynaptic reflex was 9.0 ± 4.8uM (n=7). The time courses of reflex depression and motoneurone depolarization differed, suggesting the effects might be independent. Further, 5-CT depressed reflex responses at concentrations which did not appear to depolarize motoneurones, e.g. 5-CT (10nM) depressed the monosynaptic reflex by 57.4 ± 5.9% (mean ± SEM, n=4). Neuronal uptake of 5-HT was important in determining the apparent potency of the amine. In the presence of the 5-HT uptake blocker, citalopram, 0.1 or1.0uM, the potency of 5-HT was increased about a 250-fold. The IC_{50} for depression of the monosynaptic reflex by 5-HT in the presence of citalopram was 39 ± 8nM (n=7). The IC_{50} for depression of the polysynaptic reflex was 151 ± 53nM (n=5).

On superfusion with citalopram, there was a depression of monosynaptic reflex responses. This was seen consistently using a concentration of 1uM and, usually, using a concentration of 0.1uM. That this was unlikely to be due to an action of citalopram itself was suggested by the similar effect of the uptake blocker, fluvoxamine (1uM). The depression of transmission following uptake blockade may be due to extracellular accumulation of endogenous 5-HT, since the transmission block was reversed by ketanserin, 1uM. Paradoxically, this concentration of ketanserin did not antagonize the depressant action of exogenous 5-HT on reflex responses. We conclude that 5-HT is very potent in depressing segmental spinal reflexes by an action that is unlikely to involve motoneurone depolarization.

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309P SCOPOLAMINE-INDUCED DEFICITS IN A PRIMATE OBJECT DISCRIMINATION TASK ARE REVERSED BY ONDANSETRON (GR38032F)

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Scopolamine has been widely reported to produce cognitive deficits in both man and animals, including the common marmoset (Callithrix jacchus) (Ridley *et al.*, 1984). The present studies were designed to assess the ability of the 5-HT_3 receptor antagonist, ondansetron, to reverse a scopolamine-induced impairment in the marmoset. Ondansetron has been shown to improve cognitive performance of marmosets in an object discrimination reversal task (Costall *et al.*, 1989).

Mixed sex, adult marmosets (n = 5-6) were used in this study. Throughout testing, animals were presented with a series of trials in the Wisconsin General Test Apparatus (WGTA) in which the screen was raised to reveal 2 plastic junk objects covering 2 food wells; the task was to select the food rewarded object (the left/right position of which was varied according to a pseudorandom schedule). The experimental design adopted allowed an investigation into the effects of scopolamine (0.01-0.05mg/kg s.c.) on the acquisition of an object discrimination task and the retention or reversal of this task 24hr subsequently (modified from Ridley et al., 1984).

Scopolamine (0.01-0.04mg/kg s.c.), when administered 30 min before the test of object discrimination acquisition, caused a dose-dependent increase in the number of trials required to reach criterion (9 correct responses out of 10) for example, from 8.9±2.5 in saline-treated animals to 31.7±3.6 following 0.04mg/kg scopolamine ($P<0.01$). Choice latency was also increased by scopolamine, although this only achieved significance following 0.04mg/kg scopolamine (41.9s±9.6 compared with 11.4s±1.5s saline-treated animals, $P<0.05$). 24hr following scopolamine treatment there was a trend for animals to require more trials for criterion (for the retention task) or less trials to criterion (for the reversal task). However, these changes failed to achieve significance. 0.05mg/kg scopolamine induced peripheral side-effects which were incompatible with the performance of this task.

Ondansetron (10ng/kg s.c.), administered 3 times in the 24hr preceding the acquisition of the object discrimination task, failed to reverse the effects of scopolamine (0.02mg/kg), although a reduction (not significant) in the number of trials to reach criterion was observed. However, pretreatment with ondansetron (0.1 and 1μg/kg s.c., 3 times during preceding 24hr) significantly reversed the effects of scopolamine (0.02mg/kg) on acquisition, such that the response of animals was indistinguishable from saline-treated marmosets. No effects were observed following administration of ondansetron alone.

These data provide further evidence for a role for 5-HT in the modulation of cognitive processes and thus a potential role for the 5-HT_3 receptor antagonists to improve cognitive deficits linked with a cholinergic dysfunction.

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310P THE TRH ANALOGUE, MK-771, INCREASES RAT HIPPOCAMPAL BUT NOT STRIATAL ACETYLCHOLINE RELEASE *IN VIVO*

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TRH and/or its various stable analogs have been shown to activate cortical and septohippocampal cholinergic pathways, increasing brain acetylcholine turnover (Malthe-Sorensson *et al,* 1978) and acetylcholine release in-vitro (Zuzuki *et al,* 1989). We now present evidence using intracerebral dialysis that MK-771 [L-N-(2-oxypiperidin-6yl-carbonyl)-L-histidyl-L-thiazolidine-4-carboxamide], a potent analog of TRH, increases acetylcholine release in hippocampus but not striatum of conscious rats.

Male Sprague-Dawley rats (250-350 g) were anaesthetised with pentobarbitone (60 mg/kg i.p., Sagatal, RMB) and implanted with a dialysis probe either in the hippocampus (A -5.8 mm from bregma, L 4.8 mm; V 8.0 mm from dura) or striatum (A + 0.5 mm from bregma; L 3.0 mm; V 7.5 mm from dura).

Approximately 18h later the probe was perfused with artificial cerebrospinal fluid (composition in mM, NaCl 125, KCl 2.5, $MgCl_2$ 1.18, $CaCl_2$ 1.26) containing neostigmine 2μM at a rate of 2μl/min. Samples were collected at 10 min (hippocampus) or 20 min (striatum) intervals and frozen at -70°C until required for analysis of acetylcholine essentially as described by Damsma *et al,* 1988. Basal release of acetylcholine in the hippocampus was 1.05 ± 0.11 pmol/20 μl (mean ± SEM, n = 23) and was unaffected by saline administration (1 ml/kg i.p.). The muscarinic antagonist scopolamine (1 mg/kg i.p.) caused a marked increase of acetylcholine release (2346% of basal, n = 5, $p < 0.01$). N-methyl-scopolamine (1 mg/kg i.p.), which does not readily cross the blood brain barrier, caused a slight increase (177% of basal values, n = 3). MK-771 (5 and 10 mg/kg i.p.) increased hippocampal acetylcholine release (320 and 360% of basal n = 4, $p < 0.01$, respectively) with maximal effects 40-60 min after injection. In striatum, basal acetylcholine release increased over the first two hours, reaching values of 3.16 ± 0.45 pmol/20 μl (n = 21) which then remained essentially stable and unaffected by saline administration (1 ml/kg i.p.). Scopolamine (1 mg/kg i.p.) caused a marked increase of acetylcholine release (961% of basal, n = 6, $p < 0.01$) with maximal effects at 40-60 min. However in contrast to its effect in hippocampus, MK-771 (5 mg/kg i.p.) did not significantly effect acetylcholine release when compared to saline injected rats.

This study demonstrates that systemically administered MK-771 increases hippocampal but not striatal acetylcholine release in vivo.

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311P INHIBITORY EFFECTS OF PHORBOL ESTERS ON MUSCARINIC RECEPTOR-LINKED INOSITOL PHOSPHATE ACCUMULATION AND INTRACELLULAR $Ca^{2+}{}_i$ HOMEOSTASIS IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS

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In previous studies we have shown that carbachol increases inositol phosphate (InsP) accumulation and causes a biphasic increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$), the peak phase being due to the release of stored Ca^{2+} and the plateau phase due to Ca^{2+} influx (Lambert & Nahorski 1990a). Ca^{2+} entry does not involve voltage-sensitive Ca^{2+} channels (Lambert & Nahorski 1989, and this meeting) and does not involve a pertussis toxin-sensitive G-protein (Lambert & Nahorski 1990b). In this study we confirm the inhibitory effect of phorbol esters on InsP accumulation and utilize this to determine whether Ca^{2+} entry in SH-SY5Y cells is mediated by increased InsP levels.

SH-SY5Y cells were exposed to various concentrations of phorbol dibutyrate (PDBu) for <30 mins. All experimental incubations were performed at 37°C in Krebs-Henseleit buffer, pH 7.4. InsP accumulation was measured in cells preincubated for 48 hours with 4μCi/ml $[^3H]$-inositol, and test-stimulated for 15 mins in the presence or absence of $[^3H]$-inositol and 5mM Li^+. $[Ca^{2+}]_i$ measurements were made in fura-2 loaded cells and calculated according to Grynkiewicz et al.(1985). All data are mean ± SEM (n=3). PDBu treatment inhibited carbachol (1mM) stimulated InsP (total + 5mM Li^+) accumulation in a dose-related manner. Maximum (48%) and half maximum inhibition occurring at 10^{-6}M and 4.1 ± 1.3 x 10^{-8}M PDBu respectively. PDBu (10^{-6}M) also significantly reduced carbachol (1mM) stimulated $[^3H]$-$InsP_1$ to $InsP_4$ (absence of Li^+) accumulation. Pretreatment of SH-SY5Y cells with PDBu (10^{-6}M) reduced both peak (release from internal stores) and plateau (influx) phase $[Ca^{2+}]_i$ (Table 1).

These data confirm an involvement of PKC activation in the control of InsP accumulation and could suggest a role for inositol phosphates in the mechanism of Ca^{2+} entry in SH-SY5Y cells.

Table 1: The effect of PDBu (10^{-6}M) on carbachol (10^{-4}M) stimulated rises in $[Ca^{2+}]_i$ (nM)

Condition	Basal	Peak	Plateau
Carbachol - PDBu	80 ± 9	370 ± 7	222 ± 2
Carbachol + PDBu	87 ± 8	241 ± 10*	161 ± 8*
% reduction	-	35 ± 2	27 ± 3

*p<0.002 compared with absence of PDBu.

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312P EFFECTS OF CHRONIC ADMINISTRATION OF A TRH ANALOGUE (RX77368) AND ATROPINE ON COGNITIVE BEHAVIOUR AND BRAIN BIOCHEMISTRY IN THE RAT

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Thyrotrophin-releasing hormone (TRH) enhances central cholinergic function and TRH analogues have been postulated as a therapy for Alzheimer's disease (Mellow et al., 1989) where cognitive deficits correlate with dysfunction of central cholinergic neurones. This study was undertaken to evaluate whether intracerebroventricular (i.c.v.) injection of a TRH analogue (RX77368, pGlu-His-3,3'-dimethylProNH$_2$) in the rat, could alter the cognitive deficit and biochemical effects produced by atropine. Cognitive behaviour was tested in a Morris water maze followed by analysis of brain TRH levels and choline acetyltransferase (ChAT) activity.

Male Hooded Lister rats (180-230 g) were cannulated i.c.v. under sodium methohexitone (60 mg kg^{-1} i.p.) anaesthesia and following 7 days recovery were trained on a water maze (Morris, 1981). Using an unbiased platform position rats were tested on 2 concurrent trials for 5 consecutive days, each trial lasting 180 s and all rats remained on the platform for 60 s. Four groups of rats (n=8 each) were injected 45 min prior to the first trial each day with either saline (groups A and B; 1 μl i.c.v.) or RX77368 (groups C and D; 10 μg i.c.v.) followed 30 min later with either saline (A and C; 1 ml kg^{-1}) or atropine (B and D; 6 mg kg^{-1} i.p.). After the last trial, rats were decapitated and bilateral halves of the hypothalamus, nucleus accumbens (NAC), hippocampus (HIP), septum (SEP) and parietal cortex were removed to determine TRH levels by radioimmunoassay and ChAT activity by radioenzymatic assay as described previously (Fone et al., 1988).

The time taken to find the platform significantly decreased over the 10 trials indicating learning but compared with saline controls learning in the saline/atropine group was significantly inhibited (ANOVA F ratio=10.48, P=0.006 comparing both within and between group differences) and this effect was significantly reversed (F ratio=11.9, P=0.004) in the RX77368/atropine, but not the RX77368/saline group, indicating that RX77368 could counteract the cognitive deficit produced by atropine. TRH levels in the NAC, HIP and SEP (1.1±0.2, 0.9±0.1 and 0.6±0.06 pg μg^{-1} protein, respectively) in the saline controls were significantly elevated (P<0.01, Student's t-test) by 40, 51 and 75% respectively in the saline/atropine group. Compared to saline controls, basal ChAT activity in the SEP (0.62±0.05 nmol ACh h^{-1} g^{-1} wet weight) was significantly reduced (P<0.05) by 37% in the group that received RX77368/atropine.

These behavioural and biochemical results are consistent with the proposal that TRH modulates cholinergic function in the septo-hippocampal pathway and suggest that TRH may be involved in learning and memory.

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313P NICOTINIC RECEPTORS IN THE CEREBRAL CORTEX IN ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE

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Cognitive impairment and central cholinergic dysfunction are common features of Alzheimer's disease (AD) and Parkinson's disease (PD). Degeneration of subcortico-cortical cholinergic systems and reductions in cortical pre-synaptic cholinergic markers, such as choline acetyltransferase activity, have been consistently demonstrated in AD and PD. Investigations of muscarinic cholinergic receptors in the neocortex indicate that receptor binding is unchanged in AD and increased in PD (Lange *et al.*, 1989). We have examined nicotinic receptor binding in the cortex in neuropathologically confirmed AD and PD.

Brains were obtained from 10 patients with AD and from 10 matched controls without neurological or psychiatric diseases, and from 10 patients with PD, 5 of whom had suffered from dementia, and 10 controls. The Parkinsonian patients had all been treated with L-dopa up to the time of death. Patients with AD and controls had not received any medication that is known to affect the brain. Using washed membrane homogenates we performed saturation analysis for nicotinic receptors with (-)-[^{3}H]-nicotine (concentrations 0.5 - 64 nM) in the frontal (Brodmann area 8) and temporal cortex (Brodmann area 38). Non-specific binding was defined by unlabelled nicotine.

Table 1. B_{max} values for nicotinic receptor binding (fmol/mg protein; means ± s.e.mean)

	FRONTAL CORTEX	TEMPORAL CORTEX		FRONTAL CORTEX	TEMPORAL CORTEX
CONTROLS	21.7 ± 1.3	26.9 ± 1.1	CONTROLS	23.1 ± 1.1	25.0 ± 1.1
ALZHEIMER	11.0 ± 1.4 *	11.5 ± 1.2 *	PARKINSON	12.2 ± 1.3 *	13.3 ± 0.9 *

*) Significantly different from controls ($P < 0.05$, Wilcoxon's rank-sum test)

The maximal densities of nicotinic receptors measured by specific binding of (-)-[^{3}H]-nicotine were markedly reduced in both the frontal and temporal cortex of patients with AD and demented and non-demented patients with PD. Alterations of K_D values were not observed.

The finding that nicotine stimulates the release of acetylcholine from cholinergic terminals in the cortex (Balfour, 1982) is consistent with the view that nicotinic receptors are located pre-synaptically on cholinergic axons. The present results of reduced cortical nicotinic receptor binding point to the potential for stimulation of the remaining nicotinic receptors as a treatment of the cholinergic deficit in AD and PD.

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314P DRUG MANIPULATION REVEALS DIFFERENT PATHWAYS FOR THE INDUCTION OF PURPOSELESS CHEWING AND FACIAL TREMOR IN RATS

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Previous electromyographical (EMG) investigations showed SKF 38393 and physostigmine to induce an increase in both purposeless chewing and facial tremor in rats (Collins et al, 1990). However, the results showed that while physostigmine preferentially induced purposeless chewing, SKF 38393 preferentially induced facial tremor. We now report that these facial movements can be further distinguished by treatment with SCH 23390 or atropine.

EMG signals were obtained from the right masseter muscle of male Wistar rats (300-325g at the time of surgery) as previously described (Collins et al, 1990). Rats were monitored for 35 minutes immediately following drug administration. Purposeless chewing was scored by visual observation; facial tremor was scored from the characteristic 7-10 Hz EMG signal.

Acute administration of SKF 38393 (4.0 mg/kg, sc) or physostigmine (0.1 mg/kg, ip) increased both purposeless chewing and facial tremor (Table 1). Coadministration of SCH 23390 (0.1 mg/kg, ip) had no effect on the rate of purposeless chewing in control or physostigmine treated animals, but blocked the increase in purposeless chewing induced by SKF 38393. SCH 23390 abolished facial tremor in control animals and reduced SKF 38393 and physostigmine induced facial tremor by 46% and 60% respectively, although these reductions were not significant. A high dose of atropine (25 mg/kg, ip) reduced the rate of purposeless chewing in control, SKF 38393 and physostigmine treated rats. Administration of atropine also markedly reduced facial tremor in control and physostigmine treated rats. Atropine reduced SKF 38393 induced facial tremor by 46%, but this reduction was not significant.

Table1. Effect of SCH 23390 and atropine on purposeless chewing and facial tremor induced by SKF 38393 and physostigmine.

	Purposeless Chews / 35 minutes			Facial Tremors / 35 minutes		
Treatment	Control	SCH 23390	Atropine	Control	SCH 23390	Atropine
Control	120 ± 27	101 ± 19	32 ± 14	2.5 ± 2.0	0.0 ± 0.0	0.0 ± 0.0
SKF 38393	265 ± 27*	109 ± 14$^{\Delta}$	65 ± 15$^{\Delta}$	19.2 ± 3.9*	10.4 ± 3.3	10.4 ± 4.3
Physostigmine	709 ± 66*	583 ± 72*	86 ± 23$^{\Delta}$	14.0 ± 6.7*	5.6 ± 2.0	0.4 ± 0.4$^{\Delta}$

All results are expressed as mean ± SEM; Purposeless chewing compared using Dunn's test. Facial tremor compared using Mann-Whitney U-test* $P < 0.05$, compared to control. $^{\Delta}$ $P < 0.05$, compared to treated group.

These results suggest that purposeless chewing and facial tremor can be further differentiated by their response to cholinergic and dopaminergic antagonists.

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315P QUISQUALATE STIMULATES PHOSPHOINOSITIDE METABOLISM BY INTERACTION WITH MORE THAN ONE RECEPTOR MECHANISM

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It is generally agreed that excitatory amino acids (E.E.A.'s) operate through two classes of receptors; the so called 'ionotropic' receptors which directly regulate the opening of ion channels and 'metabotropic' receptors linked to phosphoinositide metabolism and release of intracellular calcium. There is evidence that there are both ionotropic and metabotropic quisqualate (QUIS) - prefering receptors (Murphy & Miller 1989). Here we have examined the effects of this agonist on phosphoinositide metabolism in rat cortex and provide evidence that both ionotropic and metabotropic receptor activation leads to increased phosphoinositide metabolism. Rat cortical slices were prepared and labelled with [^{3}H]-inositol as described previously (Baird & Nahorski, 1986) and all experimental incubations were for 5 min unless otherwise stated. Isomers of inositol trisphosphate ($InsP_3$) were separated by hplc (Batty et al. 1989). QUIS was able to increase accumulation of the [^{3}H]-inositol phosphates in a dose-related fashion with an ED_{50} of 2.0μM. Maximal effects for QUIS were observed between 2 and 5 min but unlike the effects of the muscarinic agonist carbachol, responses decayed at later times, particularly at higher concentrations. QUIS induced increases of 31 ± 5% (n=5) and 178 ± 35% (n=5) in both the (1,4,5) and (1,3,4) isomers of $InsP_3$ respectively as well as a large accumulation of inositol(1,3,4,5)tetrakisphosphate ($InsP_4$) 725 ± 109% (n=5) inositol bisphosphate ($InsP_2$) 273 ± 50% and inositol monophosphate ($InsP_1$) 62 ± 7%. In contrast, (RS)-alpha-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), a selective ionotropic agonist only increased $InsP_1$ and $InsP_2$. Furthermore, the ionotropic antagonist 6,7-Dinitro-quinoxaline-2,3-dione (DNQX) (100μM), suppressed, but did not abolish the inositol polyphosphate responses to QUIS. Similarly, removal of extracellular calcium resulted in a much reduced response to this E.A.A.

These data indicate that QUIS can induce phosphoinositide metabolism by acting through two different but complementary mechanisms. In the absence of extracellular Ca^{2+}, stimulation of so-called metabotropic receptors leads to the generation of $Ins(1,4,5)P_3$ and its subsequent metabolism. Activation of ionotropic receptors by AMPA leads to a relatively selective increase in $InsP_2$, this resembles the effects of other depolarising stimuli or ionophores (Baird & Nahorski, 1990) and is probably a secondary effect of Ca^{2+} on phosphoinositide metabolsim. QUIS responses in the presence of extracellular calcium are a complex interaction mediated through both receptors in which Ca^{2+} entry directs the metabolism of $Ins(1,4,5)P_3$ to $Ins(1,3,4,5)P_4$. These data suggest that care should be taken in the characterisation of QUIS stimulated phosphoinositide responses and that both direct and indirect effects on phospholipase C may be distinguished by examining several inositol polyphosphates.

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316P CHARACTERIZATION OF [^{3}H]-IFENPRODIL BINDING TO THE RAT CEREBRAL CORTEX

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Ifenprodil and its structural analog SL 82.0715 ((±)-α-(4-chlorophenyl)-4-[(4-fluorophenyl)methyl]-1-piperidine ethanol) possess cytoprotective activity in animal models of focal ischaemia (Benavides et al., 1989; Gotti et al., 1988). Current data favour the hypothesis that their pharmacological activity is associated with an antagonism at the level of the polyamine modulatory site of the N-methyl-D-aspartate (NMDA) receptor complex (Carter et al., 1989). To further study the mechanism of action of ifenprodil and SL 82.0715, we characterized the binding of [^{3}H]ifenprodil to membranes from the rat cerebral cortex.

[^{3}H]Ifenprodil (spec. act. 31 Ci/mmol) labels with high affinity the σ-recognition site at 0°C (K_d = 1.93 nM) or at 37°C (K_d = 5.3 nM). Thus, [^{3}H]ifenprodil binding at 37°C is inhibited by haloperidol (IC_{50} = 24 nM), (+)-3PPP (IC_{50} = 230 nM) and di(2-tolyl)-guanidine (DTG; IC_{50}=71 nM), ifenprodil (IC_{50} = 9 nM) and SL 82.0715 (IC_{50} = 22 nM). A significant correlation exists ($p<0.001$) between the potency of 27 drugs for the inhibition of [^{3}H]ifenprodil binding at 37°C and the inhibition of [^{3}H]-(+)-3PPP binding to the σ-recognition site. At 0°C, [^{3}H]ifenprodil binding is also inhibited by (+)-3PPP (IC_{50} = 100 nM), DTG (IC_{50} = 81 nM) and GBR 12909 (IC_{50} = 5.6 nM) although these drugs only affect approximately 70% of the specific binding as defined using ifenprodil (10 μM). In the presence of 3 μM GBR 12909 to mask the σ-receptor and a putative piperazine acceptor site, a second high affinity [^{3}H]ifenprodil binding site (K_d = 36.7 nM, Bmax = 2.14 pmol/mg protein) can be demonstrated, which is inhibited by unlabelled ifenprodil (IC_{50} = 46.3 nM) and SL 82.0715 (IC_{50}= 128 nM). Under these experimental conditions, [^{3}H]ifenprodil binding is sensitive to inhibition by the polyamines spermine (IC_{50} = 8.4 μM) and spermidine (IC_{50} = 70 μM) but not putrescine (IC_{50} ≧ 1000 μM). Cationic NMDA blockers (Zn^{2+}, Mg^{2+}) and competitive NMDA antagonists (CPP, 2APV) produce a partial inhibition, suggesting a possible allosteric modulation of polyamine-sensitive [^{3}H]ifenprodil binding.

The significance of the high affinity component of [^{3}H]ifenprodil binding to the σ-recognition site remains to be established. The binding of [^{3}H]ifenprodil to a polyamine modulatory site possibly associated with the NMDA receptor confirms previous observations (Carter et al., 1989) and may be relevant to the molecular mechanism of its anti-ischaemic activity.

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317P DETERMINATION OF PLASMA AND CSF LEVELS OF MK 801 REQUIRED FOR NEUROPROTECTION IN THE QUINOLINATE-INJECTED RAT STRIATUM FOLLOWING INTRAVENOUS INFUSION OF THE DRUG

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MK-801 (dizocilpine maleate) is a potent antagonist of N-methyl-D-aspartate (NMDA) receptors which is thought to act at the associated ion channel (Wong *et al,* 1986). MK-801 is neuroprotective following intrastriatal injections of the endogenous NMDA receptor agonist quinolinate (Quin) and in cerebral ischaemia models (Gill *et al,* 1987; Foster *et al,* 1988). In this study a continuous intravenous (i.v.) infusion of MK-801 was made to determine the levels in plasma and CSF required for protection of striatal neurones following a direct injection of Quin. The Quin-injected rat striatum model (Foster *et al,* 1988) was adapted to allow the infusion of MK-801 through a cannula implanted in the external jugular vein. Cannulae were also placed in the cisterna magna and the tail vein for CSF and plasma sampling, respectively, to determine MK-801 levels (radioimmunoassay). Quin was injected into the right striatum and MK-801 given as an i.v. bolus injection 30 min later followed by a constant i.v. infusion to maintain plasma levels over a 4 hour period. Neurodegeneration was assessed by measuring changes in striatal choline acetyltransferase (CAT) and glutamate decarboxylase (GAD) activities 7 days later. Two dosing regimes of MK-801 were employed, 'low': 0.12mg/kg bolus, 1.8μg/kg/min infusion and 'high': 0.4mg/kg bolus, 6μg/kg/min infusion, which gave the steady-state plasma levels of MK-801 indicated in Table 1. In the high dose group, CSF levels of MK-801 were initially elevated (126nM) at 10 min after dosing, but fell by 120 min to a steady-state level of 91nM (Table 1), which was approximately 60% of the plasma concentration. As shown in Table 1 the low dose regime resulted in a small, but significant neuroprotective effect with respect to CAT and GAD activity whereas the high dose regime resulted in almost complete neuroprotection as assessed by both enzymes activities.

Table 1	MK-801 conc (nM)		Neuroprotection (% decrease of enzyme activity)			
	Plasma	CSF	CAT		GAD	
Saline	-	-	65.7 ± 9.4	(9)	80.3 ± 4.2	(9)
Low dose	55 ± 5.7 (9)	25 ± 5.0 (4)	41.2 ±7.0*	(13)	52.6 ± 7.3*	(13)
High dose	149 ±14.2 (6)	91 ± 13.4 (5)	5.4 ±1.9**	(6)	25.3 ± 9.4**	(6)

Values are the mean ± sem of the number of animals in parenthesis. * $p < 0.05$, ** $p < 0.01$, Duncan's test of saline controls.

In conclusion, we have defined the plasma concentration of MK-801 required for neuroprotection in the rat quinolinate injected striatum model. The steady state CSF concentration of 91nM which produced good neuroprotection correlates well with the *in vitro* affinity of MK-801 for the NMDA receptor (Wong *et al,* 1986).

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318P SIMULTANEOUS INTRACELLULAR RECORDING FROM NEURONES OF ORIGIN AND TERMINATION OF THE PERFORANT PATH DURING Mg^{++}-FREE INDUCED EPILEPTOGENESIS *IN VITRO*

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Perfusion with Mg^{++}-free medium elicits epileptiform activity in the pyramidal cells of hippocampal slices but not in dentate granule cells (DGC). However, if the entorhinal cortex (EC) is an integral part of the slice, small (0.2-0.5 mV), long lasting (10's of sec) negative field potentials can be recorded extracellularly in the dentate. These events are driven by ictal-like discharges occurring in the EC (Jones and Heinemann, 1988). In the present study we used intracellular recording to look at the Mg^{++}-free induced events in DGC, their relationship to events in the EC and the role of excitatory amino acid receptor subtypes in the propagation of activity between the two areas. Slices consisting of hippocampus and EC were cut from rat brain and maintained in vitro by conventional means. Simultaneous intracellular recordings were made from cells in layer II of the EC and DGC or from layer II and layer IV/V cells in the EC. Mg^{++}-free medium was prepared by omitting $MgSO_4$. D,L-2-aminophosphonovalerate (2-AP5, 20 uM) and 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX, 0.5-5 uM) were used as antagonists of N-methyl-D-aspartate (NMDA) and non-NMDA receptors respectively.

After 40-60 min of perfusion with Mg^{++}-free medium ictal-like epileptiform discharges began to occur in the EC. These events (Jones and Heinemann, 1988) consisted of a prolonged (up to 65 sec) depolarization (25-45 mV) superimposed with a series of afterdischarges. Events in layer II cells were generally smaller than those in layers IV/V and simultaneous intracellular recordings indicated that the events in the deep layers were driving events in layer II. In layer II-DGC paired intracellular recordings (n=15) a large (15-35 mV) depolarization occurred in the DGC 1-3 ms after initiation of the ictal-like event in the layer II cells. However, whereas the depolarization was maintained at a plateau in the EC neurones, that in the DGC declined to resting potential. Every afterdischarge on the plateau was followed (1-3 ms) by a small, discrete depolarization in the DGC. The depolarizations in the DGC rarely gave rise to action potentials. The epileptiform discharges in layer II cells were abolished in an all or none fashion by bath applied 2-AP5 and at the same time all corresponding depolarizations in the DGC disappeared. In contrast, bath applied CNQX, markedly reduced the events in the DGC with little effect on the cortical discharges. Droplet application of either 2-AP5 (0.25 ul, 80 uM) or CNQX (0.25 ul, 20 uM) to the dentate reduced the depolarizations in the DGC by around 20 and 60 % respectively but had no effect on events in the EC.

The results show that ictal-like discharges in EC can drive depolarizing events in the dentate gyrus but reaffirm the resistance of the dentate to the generation of epileptiform activity. The depolarizing responses in the DGC are mediated primarily by non-NMDA receptors with a lesser contribution from activation of NMDA receptor.

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319P HUMAN ATRIAL 5-HT RECEPTORS: SIMILARITY TO RODENT NEURONAL 5-HT$_4$ RECEPTORS

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5-Hydroxytryptamine (5-HT) increases contractile force, cyclic AMP and cyclic AMP-dependent protein kinase (cA-PrK) activity in human atria; the positive inotropic effects of 5-HT are surmountably antagonised by ICS205930 (Kaumann et al.1989). 5-HT and the gastrokinetic benzamide BRL24924 also stimulate a neuronal adenylate cyclase through ICS205930-sensitive 5-HT receptors, designated 5-HT4 by Dumuis et al (1988, 1989). The human atrial 5-HT receptor may resemble the neuronal 5-HT4 receptor (Kaumann et al.1989). We now have studied the effects of BRL24924 on right atrial appendages obtained from ß-blocker-treated patients undergoing bypass surgery. Usually 4 strips were prepared from each atrium and paced at 0.5 Hz in Krebs solution at 37°C. A single agonist concentration-effect curve was determined on each strip and the experiments terminated with 200 µM (-)-isoprenaline (ISO). In separate experiments, carried out in the presence of 400nM propranolol, one strip served as control, a second strip was exposed to 10 µM BRL24924, a third to 10 µM 5-HT and a fourth to 200 µM ISO. After 5 min, when contractile force was steady, the tissues were freeze-clamped and extracts assayed for cyclic AMP and cA-PrK activity ratio (Kaumann et al.1989).

Table 1	5-HT	n	BRL24924	n
Max. Δ force (no cocaine)(%ISO)	54±4	22	30±4	13
Max. Δ force (cocaine)(%ISO)	50±8	6	37±8	6
pEC50,M, force (no cocaine)	6.7±0.1	22	6.4±0.1	13
pEC50,M, force (cocaine)	7.4±0.2	6	6.3±0.1	6
log conc.ratio 2 µM ICS205930	1.1±0.2	5	0.7±0.2	6
Δ Cyclic AMP(%ISO)	51±13	7	34±8	7
Δ cA-PrK ratio (%ISO)	67±14	7	47±8	7

Results are $\bar{x}$ ± sem. (-)-Isoprenaline (200 µM) increased cyclic AMP from 18±3 to 134±19 pmol.mg^{-1} and cA-PrK ratio from 0.16±0.02 to 0.51±0.05 BRL24924 increased contractile force with an intrinsic activity of 0.6-0.7 relative to 5-HT. Cocaine 6µM did not affect the potency of BRL24924 but potentiated 5-HT. As expected from a partial agonist(p), BRL24924 surmountably antagonised the inotropic effects of 5-HT. The pKp (M) of 6.7±0.1 was independent of the BRL24924 concentration (0.6-6 µM). The positive inotropic effects of both BRL24924 and 5-HT were surmountably antagonised by 2 µM ICS205930. BRL24924 10 µM enhanced both cyclic AMP and cA-PrK activity.The data are consistent with the 5-HT4 nature of human atrial 5-HT receptors. 3 nM BRL24924 increases human gastric motility (Burke and Sanger, 1988) but is ineffective on atria (threshold conc 60 nM) suggesting either 5HT$_4$ receptor heterogeneity or tissue selectivity.

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320P COMPARISON OF CLASS III ANTIARRHYTHMICS AND GLIBENCLAMIDE ON ACTION POTENTIALS FROM GUINEA-PIG PAPILLARY MUSCLE UNDER NORMOXIC AND HYPOXIC CONDITIONS

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In view of the prevalence of sudden deaths arising from ventricular rhythm disturbances, there is much interest in the control of cardiac refractoriness and dispersion by drugs. Potassium channel subtypes have been recently investigated with the recognition that prolonged repolarization of the action potential via pharmacological blockade of the delayed rectifier offers a means by which the refractory period can be extended and extracellular potassium accumulation can be reduced. d-Sotalol and other highly potent blockers of this channel have been described, among them UK-66914 (Gwilt et al, 1988). Additionally, ATP-dependent potassium channels are present in ventricular muscle (Noma, 1983) and these open when intracellular ATP concentrations fall during ischaemic or hypoxic conditions. Their opening would lead to shortening of the action potential, decreased refractoriness and accumulation of extracellular potassium, all of which would predispose the heart to arrhythmias. Blockers of these ATP-dependent potassium channels, such as glibenclamide (Sanguinetti et al, 1988) would therefore be expected to have a beneficial profile in acute ischaemic situations where there is increased risk of arrhythmias.

We have compared the pharmacological profile of the Class III antiarrhythmics d-sotalol and UK-66914 with that of glibenclamide under normoxic conditions and during hypoxia. Action potentials were recorded by microelectrode impalements from guinea-pig (250-300 g) papillary muscles at 36.5°C under normoxic (Krebs bathing solution, gassed by 95% O_2/5% CO_2) or hypoxic conditions (glucose-free Krebs, gassed by 95% N_2/5% CO_2). d-Sotalol (10-100 µM) produced a modest increase in the duration of action potential repolarization (APD_{90}) under normoxic conditions whereas UK-66914 (1-100 µM) was more potent and produced a longer elongation of APD_{90} than d-sotalol. There was little or no Class III action of glibenclamide (10-30 µM) in normoxic conditions. When the bathing solution was changed to the hypoxic solution, there was a prominent shortening of APD_{90} and a small decrease in action potential amplitude which attained a steady state after 20-25 min. In agreement with earlier work (Cobbe et al, 1985), the Class III action of sotalol (30 µM) was absent under hypoxic conditions. While this could have been due to the rather weak potency of d-sotalol, a loss of activity of the highly potent UK-66914 (30 µM) was also observed under hypoxic conditions. In contrast, glibenclamide (10 µM) partially restored the hypoxic APD_{90} in a time-dependent manner.

These data may have implications for clinical situations where there has been severe myocardial ischaemic damage. Tissue heterogeneity and a differing pharmacological profile of Class III compounds under these conditions might necessitate a more appropriate therapy for the control of some ventricular arrhythmias.

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321P BDF 9148: A cAMP-INDEPENDENT POSITIVE INOTROPIC AGENT THAT ACTIVATES SODIUM CHANNELS WITHOUT PROLONGING Q-T DURATION

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The ideal agent for long term treatment of congestive heart failure is not yet available. Biochemically, the ideal inotropic agent should not elevate intracellular cAMP content and - electrophysiologically - not affect cardiac action potential duration (APD). The discovery of DPI 201-106 (DPI), a sodium channel activator by Scholtysik et al. (1985), was considered a major breakthrough in the search for a substitute to the cardiac glycosides, but the electrophysiological profile of DPI greatly limited its clinical use. By prolonging the inactivating sodium current, DPI causes a marked prolongation of cardiac APD resulting in a lengthening of the Q-T interval of the ECG in man (Rüegg et al., 1987). We present a new sodium channel activator with an electrophysiological profile, critically different from DPI.

BDF 9148, 3-(1-diphenylmethyl-acetidine-3-oxy)-2-hydroxy-propoxy)-1H-indole-2-carbonitrile (BDF), increased force of contraction in electrically driven (1 Hz) guinea pig papillary muscles in a concentration dependent fashion giving rise to an IC50 value of 0.6 µM (equipotent with DPI). Bradycardia was 10-fold less pronounced under BDF. Both BDF and DPI had no influence on cAMP content in cardiac tissue even at maximal inotropic concentrations (10 µM each). Similar to DPI, the inotropic effect of BDF was not affected by treatment with propranolol, prazosin, cimetidine or carbachol (10 µM each). Treatment with tetrodotoxin completely abolished the inotropic effect of both drugs, implying sodium channel dependence of the cardiotonic effects. In contrast to DPI, which produced an irreversible prolongation of APD, BDF only caused a transient prolongation of APD in guinea pig papillary muscle (in conventional electrophysiological experiments using glass electrodes). At 3 and 10 µM, DPI irreversibly prolonged APD90 by 25.2 ± 7.41 and 69.2 ± 8.9 ms, respectively (means$\pm$SEM, n=6; control APD90=177 ± 5.2 ms). BDF affected APD in a biphasic manner: An initial prolongation phase lasting 5-15 min was followed by reversal of prolongation at 30-90 min, such that APD90 was no longer significantly different from duration in controls after 90 min. In anesthetized dogs, BDF doses of 0.1-3 mg/kg i.v. caused a dose-dependent increase of leftventricular dp/dtmax from control values of 2100 ± 400 to 6550 ± 620 mmHg/sec. While BDF and DPI were equieffective in elevating dp/dtmax, only DPI lowered total peripheral resistance and heart rate. Similar to its effects on APD, DPI prolonged the Q-T duration from 242 ± 2 to 285 ± 10 ms at 3 mg/kg i.v. In contrast, BDF doses of 0.1-10 mg/kg did not affect Q-T duration, nor did BDF affect Q-Tc (Bazzet). In conclusion, BDF 9148 constitutes a significant advance in the quest for a substitute to the cardiac glycosides.

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322P ANTAGONISM OF ADENOSINE P_1 AND MUSCARINIC RECEPTOR ACTIVATION OF ^{86}RUBIDIUM EFFLUX FROM GUINEA-PIG LEFT ATRIA

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Adenosine P1- and muscarinic-receptor agonists produce negative inotropy in left atria (LA) by activating common K^+ channels (Kurachi et al. 1986). Bromobenzoyl-methyladamantylamine (BMA) antagonizes the electrophysiological effects of P1 and muscarinic-agonists in LA, possibly by blockade of these common channels (Meszaros et al. 1982; Meszaros et al. 1984). We have examined the effects of adenosine (AD), R-PIA (PIA) and carbachol (CAR) upon ^{86}Rb efflux, an indicator of changes in K^+ efflux, and their modification by the K^+-channel antagonists BMA, 4-aminopyridine (AP) and by 8-phenyltheophylline (PT).

Isolated paced LA (2Hz, 5ms, threshold + 50%) were set up in 4mls of Krebs bicarbonate solution (Krebs) at 37.5°C, gassed with 5% CO_2 in O_2. When AP was used guinea-pigs were reserpinized (5mgkg^{-1} ip, 24 hrs before). LA were incubated in Krebs containing ^{86}Rb 37mBq1^{-1} and then washed every 2 mins for 120 mins. Each 4ml sample was counted in the Cerenkov mode. Radioactivity remaining in the LA was measured. A single concentration-response curve for an agonist was constructed by exposing the LA to four increasing concentrations (4 x 2 mins each) starting 60 mins from the end of loading. Antagonists were present continuously from 20 mins before agonist exposure. Mean changes in the rate constant for Rb efflux (K) were calculated ($n\geq4$).

		Control	PT (10µM)	BMA (100µM)	AP (10mM)
Maximum	AD (2.25mM)	0.00389	0.00255*	0.00185*	0.0011*
Increase	PIA (15.6µM)	0.00223	-	0.00101	0.00015*
in K(min^{-1})	CAR (11µM)	0.00400	-	0.00179*	-

*Significant ($p<0.05$) difference from control value.

In the absence of drugs, there was no significant change in K during the experiment. AD, PIA and CAR induced significant dose related increases in K. Alone BMA significantly reduced K from 0.0104 to 0.0087 min^{-1}, presumably due to blockade of active K^+ channels, however AP and PT did not. PT caused parallel rightward shift of the AD curve and reduction of the response to the highest concentration, consistent with AD increasing K via a P1-receptor. AP greatly reduced the responses to PIA and AD suggesting that AP blocks the P1-linked K^+-channel in LA. BMA "flattened" the curves for AD, CAR and PIA with a significant reduction in response to the highest AD and CAR concentrations. This suggests that BMA interacts with common K^+-channels linked to P1 and muscarinic receptors.

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323P MYOCARDIAL ENERGY PRODUCTION IN RATS WITH ACUTE RENAL FAILURE

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Previous studies have demonstrated reduced positive inotropic responses of isolated ventricular tissue from rats with acute renal failure (ARF) to a range of stimulants (isoprenaline, IBMX, BAY K 8644 and Ca^{2+}) which act at different levels within the myocardium (Robinson et al., 1989). Such non-selective depression of inotropic responsiveness suggests a fundamental defect within the heart, possibly at the level of energy production or utilisation.

This study has investigated myocardial energy production by determining the respiration of mitochondria from hearts of rats 48 hours after induction of ARF produced by i.m. injection of 10 $ml.kg^{-1}$ 50% v/v glycerol in 0.9% saline. Mitochondria were isolated according to the method of McCormack & Denton (1988). State 3 (ADP-stimulated), state 4 (resting) and DNP-uncoupled respiratory rates were measured in the presence of a range of substrates which supplied electrons at each of the three proton-pumping sites of the respiratory chain. Respiratory control (RCR) and ADP:0 ratios were calculated. There was a significant reduction ($P < 0.05$) in ADP:0 ratios of mitochondria from rats with ARF compared with those from controls in the presence of both NADH-linked substrates (control 2.2 ± 0.1, n = 7; ARF 1.7 ± 0.1, n = 7) and $FADH_2$-linked substrates (control 1.3 ± 0.1; ARF 1.0 ± 0.1). However, respiratory rates and RCRs of mitochondria were not significantly different ($P > 0.05$) between control and ARF rats.

Adenine nucleotide and creatine phosphate levels in hearts of rats with ARF were measured according to the method of Hammer et al., (1988). Nucleotides were extracted with perchloric acid and samples assayed by HPLC with detection at 214 nm. There was no significant difference in levels of ATP, ADP, AMP, creatine phosphate (CrP) or in the cellular energy charge (EC) between control and uraemic animals (Table 1).

	ATP	ADP	AMP	CrP	EC
C	21 ± 4	4.4 ± 0.6	0.51 ± 0.05	15 ± 2	0.896 ± 0.005
ARF	23 ± 2	4.9 ± 0.5	0.58 ± 0.06	15 ± 3	0.895 ± 0.005

Table 1 Adenine nucleotide levels, creatine phosphate levels and energy charge of myocardia from glycerol-injected rats (ARF) and controls (C); n = 8. All levels given as $nmol.mg\ myocardial\ protein^{-1}$. Values are mean ± s.e. mean.

These results show an uncoupling of oxidative phosphorylation although the degree of uncoupling appears to have no impact on cellular energy balance. This suggests that the defect responsible for reduced inotropic responsiveness in ARF does not lie in energy production and thus may result from impaired energy utilisation.

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324P PLATELET AGGREGATION AND THE EFFECTS OF ROLIPRAM ON ISCHAEMIA- AND REPERFUSION-INDUCED ARRHYTHMIAS IN ANAESTHETIZED RABBITS

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We have shown previously that the nonselective phosphodiesterase (PDE) inhibitor isobutylmethylxanthine (IBMX) reduced platelet aggregation and ischaemia-induced arrhythmias in anaesthetized rabbits whereas the selective PDE III inhibitor milrinone had greater antiplatelet but less antiarrhythmic activity (Holbrook & Coker, 1989). We have now examined the effects of the PDE IV inhibitor rolipram and investigated the PDE enzymes found in rabbit platelets.

Male NZW rabbits (1.9 to 3.2 kg) were anaesthetized and prepared for coronary artery occlusion as described previously (Coker, 1989). Rolipram 30 $\mu g\ kg^{-1}$ + 3 $\mu g\ kg^{-1}\ min^{-1}$ or 100 $\mu g\ kg^{-1}$ + 10 $\mu g\ kg^{-1}\ min^{-1}$ or vehicle (25% PEG) n=10 per group, was administered i.v. After 10 min the left circumflex coronary artery was occluded for 20 min followed by 10 min reperfusion. At the end of each experiment blood was removed, platelet rich plasma prepared and platelet aggregation studied *ex vivo*.

Rolipram significantly increased heart rate (e.g. high dose from 273±8 to 316±12 beats min^{-1}) and left ventricular dP/dt_{max} (from 3360±100 to 3840±130 mmHg s^{-1}) and decreased diastolic blood pressure (from 45±4 to 38±2 mmHg). Mortality due to ventricular fibrillation during ischaemia or reperfusion was increased from 30% in controls to 70% with low dose and 80% with high dose rolipram (P=0.179 and P=0.070, Fisher's exact test) respectively. Aggregation stimulated by ADP, collagen, thrombin, and arachidonic acid, and the antiaggregatory effects of isoprenaline and prostacyclin in the presence of ADP, were similar in platelets from control and rolipram treated rabbits. Rolipram, *in vitro*, was relatively inactive on platelets from untreated rabbits, whereas milrinone potentiated the actions of isoprenaline and prostacyclin. Three types of PDE activity were observed in a soluble extract from untreated platelets after separation by ion-exchange chromatography on Mono Q FPLC. PDE I was cyclic GMP selective; PDE II was nonselective and its cyclic AMP activity was increased by cyclic GMP (1μM); and PDE III was cyclic AMP selective, with cyclic AMP hydrolysis being inhibited by cyclic GMP (1μM). The rolipram-sensitive (PDE IV) enzyme was not detected. PDE III was selectively inhibited by milrinone (IC_{50} 0.5μM) but was only weakly inhibited by rolipram (IC_{50} >250μM).

In conclusion, rolipram, unlike IBMX and milrinone exacerbated arrhythmias. Rolipram had no effect on platelet aggregation, probably due to a lack of PDE IV in rabbit platelets.

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325P DIPHENYLENE IODONIUM, A POTENT INHIBITOR OF FREE RADICAL PRODUCTION, POSSESSES ANTI-PLATELET ACTIVITY AND INTERACTS WITH MODULATORS OF PLATELET CYCLIC NUCLEOTIDES

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The aggregation of blood platelets and their surface-adhesive properties are important in haemostasis and diseases such as atherosclerosis. In common with most aerobic cells, platelets can generate oxygen-derived free radicals (Marcus, 1979). We have recently demonstrated that the superoxide anion (O_2^-) can enhance both platelet adhesion and aggregation (Salvemini et al.,1989a). Here we report that diphenylene iodonium (DPI), a potent inhibitor of free radical production in neutrophils (Cross et al., 1986), is also a potent inhibitor of platelet aggregation in human platelet rich plasma (PRP) or washed platelets (WP).

Human WP or PRP treated with indomethacin (10μM) were preincubated for 2 min (37°C, 1000 rpm) in an aggregometer. Thrombin (40-80mU/ml) or ADP (6μM) were added to the platelet suspension and the change in light transmission was monitored for a further 3 min. Iloprost (Ilo; 0.03-0.1ng/ml), sodium nitroprusside (NaNp; 2.5-5μg/ml), endothelial cells on beads (EC; 10μl) and/or DPI (0.5-2μM), were added to the platelet suspension 1 min before thrombin or ADP stimulation. DPI (0.25-2μM) inhibited in a dose-dependent manner platelet aggregation in WP with 0.5μM producing a 24±5% (n=4, P<0.001) inhibition and with 2μM a 99±1 % inhibition (n=4, P<0.0005). Similar results were also obtained in PRP (n=3). The inhibitory effect of DPI was not related to a stimulation of the platelet guanylate or adenylate cyclase, as no changes in the levels of cGMP or cAMP were observed (from 61±3 in the absence of DPI and 60±6 fmol cGMP/100μl in the presence of 2μM DPI, n=4, P<0.4 and 303±59 in the absence of DPI and 307±52 fmol cAMP/100μl in the presence of 2μM DPI, n=4, P<0.2).

In addition at low concentrations (0.5μM), DPI synergised to inhibit platelet aggregation with Ilo (n=4, P<0.005), NaNp (n=4, P<0.005) or EC (n=4, P<0.005).

In neutrophils, DPI inhibits the release of oxygen-derived free radicals through an action at the level of the NADPH-oxidase (Cross et al., 1986). As a result both the extra- and intra-cellular release of free radicals is blocked. This may explain why DPI is a) more potent than SOD at inhibiting platelet aggregation induced by high concentrations of thrombin and b) why it is also effective in PRP (Salvemini et al., 1989b). These results reinforce the importance of free radicals as pro-aggregatory agents (Salvemini et al, 1989a) and suggest that agents which prevent both the extra- and intra-cellular release of free radicals would be more effective anti-platelets drugs than those preventing only their extracellular release. The potential use of free radical scavengers as anti-platelet drugs is strengthened by their synergism with other anti-platelet agents such as NaNp or Ilo.

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326P EFFECTS OF GLYCERYL TRINITRATE AND CULTURED VASCULAR SMOOTH MUSCLE CELLS ON PLATELET AGGREGATION

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Vascular endothelial cells have recently been shown to synthesise nitric oxide (Palmer *et al.*, 1987). Organic nitrates such as glyceryl trinitrate (GTN) may be metabolised in cell-free systems to produce nitric oxide, and it has been suggested that nitric oxide production underlies their dilator action (Schröder *et al.*, 1985). Nitric oxide in addition has potent anti-aggregatory and anti-adhesive actions on platelets, and it is possible that metabolism of organic nitrates by the vascular wall results in inhibition of platelet function by production of nitric oxide. It was the purpose of this study to examine the ability of vascular smooth muscle cells to inhibit platelet aggregation in the presence of glyceryl trinitrate. Cells derived from human uterine artery were cultured to confluence in 12-well plates as previously described (Ritter *et al.*, 1990) and suspended in phosphate-buffered saline (final volume 2 ml/well) using trypsin. Aggregation of human platelets was studied using stirred platelet-rich plasma at 37 °C with aspirin (1 mM to inhibit prostacyclin production) in a Payton aggregometer. Aggregation was measured as % change in light transmission 2 min after addition of agonist; 100 % was taken as the light transmission with platelet-poor plasma. GTN and/or cell suspension was added 15 seconds before agonist. U46619 (2 μM) alone caused 73 ± 3 % aggregation (mean ± sem, n = 8). This was unaffected by addition of GTN (225 μM; 67 ± 6 %) or by a suspension of vascular smooth muscle cells (100 μl; 66 ± 5%). When both GTN and cell suspension were added together at the same dose, however, there was almost complete inhibition of platelet aggregation with U46619 (4 ± 4 %). This effect was inhibited by haemoglobin (5 μM; 49 ± 7 %) and was less marked (aggregation 59 ± 2 %) in cells previously made tolerant (as shown by a reduced ability to generate nitrite from GTN) by pre-incubation with GTN (225 μM) for 24 hours. These results show that human cultured vascular smooth muscle cells inhibit platelet aggregation in the presence of GTN, and that this effect is likely to be due to the generation of nitric oxide.

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327P EFFECT OF NITRO-ARGININE METHYL ESTER ON RENAL FUNCTION IN THE ISOLATED PERFUSED RAT KIDNEY

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Evidence for the presence of endothelium-derived relaxing factor (EDRF) in the isolated perfused rat kidney (IPRK) has recently been obtained from experiments studying the effect of EDRF inhibitors on cGMP release and renal vascular resistance (Burton et al 1990). The arginine analogue, nitro-arginine methyl ester (l-NAME), has recently been proposed as an inhibitor of the arginine-nitric oxide pathway (Collier & Vallance 1989). In the present experiments we have studied the effect of l-NAME on renal function in a model of the isolated rat kidney perfused at constant pressure to measure the glomerular filtration rate and tubular function in addition to vascular resistance.

Male Wistar rats (400-450g) were anaesthetised with sodium pentobarbitone 60mgkg^{-1} i.p. and the right ureter cannulated for the collection of urine. The right renal artery was cannulated via the superior mesenteric artery and perfusion started in situ to prevent renal ischaemia. The kidney was isolated and perfused at constant pressure (100mmHg), using a servo-controlled perfusion pump, with oxygenated Krebs-Henseleit (37°C) containing 6.7% albumin and 14mM mixed amino acids. Renal perfusate flow was monitored continuously and following a 30 minute equilibration period, [^{14}C] inulin clearances were measured together with solute excretion. The dose of l-NAME employed was determined from vasodilator experiments using the rat aortic ring.

In control experiments (n=6) renal function was stable over a 40 minute period; the renal perfusate flow was 36.7 ± 4.6 ml/min/g (0-10min) compared to 37.0 ± 5.2 ml/min/g (30-40 min); [^{14}C] inulin clearance was 0.54 + 0.05 compared to 0.53 ± 0.05 ml/min/g and sodium reabsorption 87.6 + 1.5 compared to 86.2 ± 2.1%. L-NAME (n=6), added at 20 min to give a concentration in the renal perfusate of 100μM, produced a significant fall in renal perfusate flow (P<0.001) from 44.3 ± 2.6 ml/min/g (0-10 min) to 20.6 ± 2.6ml/min/g (30-40 min); in [^{14}C] inulin clearance from 0.61 ± 0.12 to 0.30 ± 0.09 ml/min/g (P<0.01) and in sodium reabsorption from 88.5 ± 2.7 to 76.7 ± 5.2% (P<0.02).

The results indicate that in the IPRK, nitric oxide synthesis may contribute to renal vascular resistance and could be responsible for the high perfusate flow rate seen in this preparation. Independent effects of nitric oxide on either the glomerular filtration rate or the tubular handling of sodium cannot be assumed from this experiment since the decrease in sodium reabsorption and [^{14}C]inulin clearance following l-NAME could be a consequence of the increase in vascular resistance.

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328P EDRF AND VASCULAR RESPONSIVENESS IN THE PERFUSED KIDNEY OF THE SPONTANEOUSLY HYPERTENSIVE RAT

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Conflicting evidence has been obtained (Konishi & Su 1983) about the involvement of EDRF in the elevated blood pressure of the spontaneously hypertensive rat (SHR). In this study, we have attempted to determine whether there is a reduction in either endothelium dependent or independent vasodilatation in the SHR rat, using the isolated perfused rat kidney preparation.

Male spontaneously hypertensive rats (SHR) and male Wistar Kyoto rats (WKY), aged 10-12 weeks, were anaesthetised with pentobarbitone sodium (60mgkg^{-1} i.p.). The right carotid artery was cannulated and blood pressure monitored. The left renal artery was cannulated, the kidney isolated and perfused with Krebs-Henseleit solution (37°C) at a rate of 4.75 mlmin^{-1}. Single exposure concentration-response curves were performed to methoxamine. Following preconstriction with 3uM methoxamine (ED80) single exposure concentration-response curves were carried out to acetylcholine (ACh) (0.01-1μM), sodium nitroprusside (SNP) (0.01-10μM) and the calcium ionophore A23187 (0.01-0.3μM). Each vasodilator was examined in a separate group of kidneys.

SHR's demonstrated a mean systolic arterial blood pressure of 186.8 ± 3.3 mmHg (n=37) which was significantly greater (P<0.001) than that measured in WKY's at 100.7 ± 5.1 mmHg (n=30). SHR rats were also significantly heavier (P<0.01) at 293.4 ± 3.2g than WKY's at 264.9 + 5.2g. Kidney wet weights were not significantly different between the two groups before or after perfusion. There was no significant shift in the concentration-response curve to methoxamine in SHR or WKY kidneys however in WKY's the maximum increase in perfusion pressure produced by methoxamine was significantly lower (P<0.001) at 158 ± 6.8 mmHg (n=6) than that observed in SHR's at 235 ± 6.2 mmHg, (n=7). No significant differences were observed between the concentration-response curves for ACh, SNP or A23187 obtained in kidneys taken from either SHR or WKY rats.

The results of this study do not provide evidence to support a loss or a reduction in the sensitivity of the kidney to endothelium-dependent vasodilatation in the SHR. However, the study did demonstrate an increase in renal vascular contractility to methoxamine in agreement with previous findings for other contractile agents which have been investigated in a variety of vascular tissues taken from the SHR (Luscher et al 1987, Smeda et al 1988).

G.A.Burton is an SERC student.

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329P COMPARISON OF THE REGIONAL HAEMODYNAMIC PROFILES OF RO 31-6930, CROMAKALIM AND NIFEDIPINE USING PULSED DOPPLER FLOWMETRY IN THE ANAESTHETISED NORMOTENSIVE RAT

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The in vitro pharmacology of the K-channel opener, cromakalim (CK), differs from that of organic Ca entry blockers (Cook et. al., 1988). Recent studies in the cat suggest that differences may also exist in the regional haemodynamic profile of these compounds (Clapham & Longman, 1989). In the present study we compared the regional haemodynamics of the novel K-channel opener Ro 31-6930 (Paciorek et. al., 1990) with those of CK and nifedipine, in the anaesthetised normotensive rat.

Arterial pressure was recorded in male normotensive rats anaesthetised with sodium pentobarbitone (60mg/kg). Blood flow was monitored by miniaturised pulsed Doppler flow probes placed around the renal, superior mesenteric and iliac arteries (Haywood et. al., 1981). Mean vascular resistance was derived from arterial pressure/mean Doppler shift signal and the % change in this resistance produced by cumulative i.v. administration of each agent was determined. Data were analysed using split plot analysis of variance. Results given are mean ± s.e.mean (n=7).

Resting mean arterial pressure was 119.5 ± 2.6mmHg. CK (3-300μg/kg), Ro 31-6930 (1-300μg/kg) and nifedipine (100-300μg/kg) evoked maximum observed falls in pressure of 63.8 ± 4.6, 74.1 ± 4.7 and 44.3 ± 4.7 mmHg, respectively. Reductions in renal vascular resistance were most marked with CK (maximum fall; 53.6 ± 15.6%) and occurred at both sub-hypotensive (1μg/kg) and hypotensive doses. Smaller reductions in resistance were observed with sub-hypotensive and hypotensive doses of nifedipine (10-300μg/kg) and with Ro 31-6930 (3-30μg/kg). Mesenteric vascular resistance was significantly reduced (maximum falls; 50-60%) by all three agents, although CK was effective only at higher doses (10-100μg/kg). Although iliac vasculature exhibited the greatest falls in resistance with all agents (maximum falls; 60-80%), these effects were only seen at high doses. The order of selectivity of each agent for the three vascular beds is shown below. 1)CK: renal > mesenteric = iliac, 2)Ro 31-6930 and nifedipine : mesenteric > renal > iliac.

The notable selectivity of CK for the renal vasculature has previously been shown in the cat (Clapham & Longman, 1989) although not at sub-hypotensive doses as observed in the present study. Clearly, differences in haemodynamic profile exist between Ca antagonists and K-channel openers, mainly regarding their potency. The two K-channel openers tested exhibit different profiles in terms of their regional effects, possibly reflecting the structural differences between pharmacophores of the two compounds.

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330P DOES PROGESTERONE-EVOKED SUPPRESSION OF HUMAN INTRACRANIAL ARTERY CONTRACTILITY INVOLVE POTASSIUM CHANNEL ACTIVATION?

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Observations that the contractile responses of human isolated vascular muscle to low doses of K^+ (≤ 30mM) are inhibited more readily by progesterone than those evoked by higher concentrations of the cation (≥90mM) or by noradrenaline, raise the possibility that progesterone may activate K^+ channels (Leathard & Eccles, 1987). This hypothesis has now been tested by comparing the effects of progesterone with those of cromakalim on human intracranial artery (ICA) strips. Helically-cut ICA strips (1-3 days post-mortem) were suspended, counter-balanced by 0.5g, in isolated organ baths at 37°C containing Krebs' solution gassed with 95% O_2 and 5% CO_2 (pH 7.4), and allowed to equilibrate until the agonists caused reproducible contractions. Control concentration-response curves were recorded using 5-HT (60nM, 600nM, 6μM) and KCl (5, 10, 20, 40, 80mM added to 5.9mM K^+ in the Krebs' solution). Strips were then incubated for 1h with progesterone (4μM), its solvent propylene glycol (1:10,000), cromakalim (4μM) or its solvent ethanol (1:5,000). Agonist concentration-response curves were obtained in the continued presence of these agents. Each strip was then equilibrated for 20m with tetraethylammonium (TEA 1mM) and the agonist concentration-response curves were re-examined. Statistical comparisons utilized Students 't' tests. Muscle tone (changes measured as %ages of the maximum control contraction evoked by KCl) was lowered by cromakalim (-46±14%, n=10, $p<0.01$) and sometimes by progesterone (-12±14%, n=9, $p>0.05$) or propylene glycol (-13±10%, n=6, $p>0.05$) but not by ethanol (0±5%, n=10, $p>0.05$). These changes in tone were incorporated into the concentration-response curves by measuring contractions relative to the stable control baseline tone. The magnitude of the contractions evoked by 5 or 10mM KCl was reduced by progesterone (n=9, $p<0.05$) and by cromakalim (n=8, $p<0.05$) but 40 or 80mM KCl-evoked contractions were unaffected. The response to each concentration of 5-HT was attenuated by cromakalim (n=8, $p<0.05$) but was unaffected by progesterone (n=10, $p>0.05$). TEA raised the tone to approximately 50% of the previous maximum KCl-evoked contraction (n=6-9, $p<0.01$ in each of 4 groups). Superimposed concentration-response curves for KCl or 5-HT were shifted leftward and reached higher maxima than control curves when the intervening treatment was cromakalim, progesterone or ethanol ($p<0.01$ for 5-HT 60nM-6μM and $p<0.05$ for KCl 5 or 10mM but not 40 or 80mM). Following propylene glycol, TEA increased contractile responses to the lower concentrations of the 5-HT (n=6, $p<0.05$) but had no significant effect on the KCl curve (n=6). Thus, in human ICAs, potassium channel activity clearly influenced muscle tone and contractile response to 5-HT or 5 or 10mM added KCl. Progesterone suppressed only 5 or 10mM KCl-evoked contractions, indicating a more limited activation of potassium channels than elicited by cromakalim.

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331P COMPARISON OF CROMAKALIM-, PINACIDIL- AND NICORANDIL-INDUCED RELAXATION OF RAT ISOLATED BASILAR ARTERY

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Since cromakalim (CROM) was essentially inactive against KCl precontraction of rat isolated basilar artery (BA) (Parsons et al, 1990) the actions of CROM on 5-hydroxytryptamine (5-HT) spasm were assessed and compared to nicorandil (NIC) and pinacidil (PIN).

Endothelium intact rat BA was set up for isometric recording of tension (Parsons et al., 1990). Concentration-effect (C-E) curves to CROM (0.1 uM - 100 uM), PIN (0.1 uM - 100 uM) or NIC (1 uM - 500 uM) were then constructed on either elevated tone to 1 uM 5-HT and repeated in the absence or presence of either glibenclamide (GLIB) (0.1 - 1.0 uM), tolbutamide (TOL) (10 uM) or methylene blue (MB) (10 uM). C-E curves to NIC and PIN were also formed on 50 mM KCl spasm.

MB but not GLIB or TOL produced a variable contraction of resting tone. No effect was observed against 5-HT spasm for any of these compounds. CROM, PIN and NIC induced concentration dependent relaxation as shown in % of 5-HT and KCl spasm:

	5-HT spasm		50 mM KCl spasm	
	$-\log EC_{50}$ ± s.e.mean	E_{max} ± s.e.mean	$-\log IC_{30}$ ± s.e.mean	E_{max} ± s.e.mean
CROM	6.35 ± 0.04*	99.4 ± 0.9	(4.67 ± 0.24[a])	12.0 ± 5.0[a]*
NIC	4.19 ± 0.09*	91.4 ± 3.6*	3.63 ± 0.18*	42.6 ± 3.1*
PIN	5.88 ± 0.11	102.3 ± 3.2	4.88 ± 0.05	91.9 ± 2.4

[a] -log EC_{50} value on 50 mM KCl spasm (Parsons et al., 1990); n = 5-18; * $p < 0.05$ from pinacidil (Duncan's multiple range test).

TOL had no effect against CROM, PIN, or NIC induced relaxation. GLIB produced concentration related blockade of CROM, NIC and PIN with respective pA_2 values ± s.e.mean (van Rossum, 1963), with the slope of the Schild regression ± s.e.mean (Arunlakashana and Schild, 1959) given in parenthesis, were: 6.84 ± 0.20 (1.10 ± 0.20), 6.60 ± 0.14 (1.10 ± 0.40), and 6.78 ± 0.11 (0.79 ± 0.21). MB (10 uM) produced inhibition of CROM (0.1 uM - 100 uM), NIC (1 uM - 500 uM) and PIN (0.1 uM - 100 uM) induced relaxation ($p < 0.05$; Duncan's multiple range test).

This study shows that CROM induces relaxation by a similar mechanism as demonstrated in other cerebral blood vessels (Parsons et al, 1990). NIC and PIN appeared to produce relaxation by opening of K^+ channels and an additional mechanism. Interestingly, MB (10 uM) inhibited NIC, CROM and PIN induced relaxation and also blocks ACh induced relaxation of rat BA (Mackert et al. 1990).

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332P PHARMACOLOGICAL PROFILE OF TALUDIPINE (GR53992B), A NEW LONG-ACTING CALCIUM ENTRY BLOCKER

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Taludipine (T), (GR53992B (E) -4 - (-2- (-3- (-1, 1-Dimethyl-ethoxy) - 3 - oxo - 1 - propenyl)phenyl -1,4 dihydro- 2- dimethyl - amino-methyl-6 - methyl - 3,5 pyridinedicarboxylic acid,diethyl ester hydrochloride),is a new long-lasting 1,4-dihydropyridine calcium entry blocker (CEB).
In vitro T induced a potent and selective CEB activity on vascular preparations (Table 1). In addition,the effect of T was not reversed after 9 hours of drug washout in rabbit ear artery. On electrically driven guinea pig papillary muscle T induced a negative inotropic effect at concentrations at least 100 times higher than the vascular-effective ones (IC50 = 7.8 (2.9-21.0) uM).
Unlike some calcium entry blockers, T exhibited antioxidant properties in a number of in vitro tests,such as the peroxidation of rat cerebral cortical membranes (IC_{50} = 72 ± 2 uM versus 554 ± 45 uM,460 ± 104 uM and 260 ± 43 uM for Nifedipine, Verapamil and Nimodipine respectively).

Table 1

	Calcium antagonism		Relaxation
In vitro preparations	pA_2	slope	IC_{50}(nM)
Rabbit Ear Artery	8.0 (7.8-8.1)	1.0 (0.7-1.4)	
Rat Aorta			0.62(0.39-0.99)
Rat Colon			5.3 (3.7-7.5)
Rat Bladder			8.2 (6.4-10.4)

In spontaneously hypertensive rats (SHR) T induced a potent, slow onset and long-lasting blood pressure reduction (ED25% = 0.72(0.63-0.81) mg/Kg p.o. and 0.06(0.04-0.07) mg/Kg i.v. of duration greater than 9 and 3 hours respectively). Concomitant with the blood pressure reduction a slight and short-lasting heart rate increase was detected.

In one-clip two-kidney renal hypertensive dogs (RHD) T confirmed its antihypertensive properties after both oral and intravenous administration (ED25% = 0.45(0.39-0.51)mg/Kg p.o. and 0.075(0.053- 0.107) mg/Kg i.v.). The compound administered once a day for 5 consecutive days in SHR and RHD did not show any evidence of tolerance.

In anaesthetized open-chest dogs (10-100 ug/Kg i.v) long-lasting peripheral vascular resistance reductions and coronary blood flow increases were observed.

In summary, T demonstrates some beneficial properties of this class of compounds in causing peripheral and coronary vasodilation and in addition is endowed with antioxidant properties which might be useful in reducing the damage by ischaemic conditions.

333P FORMATION OF NITRIC OXIDE-LIKE ACTIVITY FROM L-ARGININE IN RAT MAST CELLS

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Nitric oxide (NO) which accounts for the biological activity of endothelium-derived relaxing factor relaxes vascular smooth muscle and inhibits platelet aggregation (Palmer et al., 1987). Besides endothelial cells, a NO-like factor is released from activated mouse macrophages (Salvemini et al., 1989a) and human neutrophils (Salvemini et al., 1989b). Here we demonstrate the release of a NO-like factor from rat serosal mast cells as determined by inhibition of platelet aggregation and elevation of cGMP levels.

Human washed platelets treated with indomethacin (10μM) were prepared as described previously (Radomski et al., 1983). Isolated rat serosal mast cells were obtained with Ficoll centrifugation gradients (Blandina et al., 1980) and resuspended in Krebs' buffer containing indomethacin (10μM). A suspension of washed platelets was incubated for 2 min at 37°C in an aggregometer with continuous stirring at 1000rpm and then stimulated with submaximal concentrations (40-60mU/ml) of thrombin. The appropriate numbers of mast cells with or without superoxide dismutase (SOD; 60U/ml) and/or oxyhaemoglobin (oxyHb; 10μM) were added to the platelet suspension 1 min before thrombin stimulation. When required the mast cells were preincubated for 60 min with the L-arginine antagonist N^G-monomethyl-L-arginine (LMA; 300μM) in the presence or absence of L-arginine (L-arg; 100μM) or D-arginine (D-arg; 100μM).

Incubation of mast cells (2-5 x 10^4) with human platelets induced an inhibition of platelet aggregation related to cell numbers, with 5 x 10^4 cells inducing a 100% inhibition (n=3, P<0.005). The inhibition induced by 2 x 10^4 cells was significantly potentiated in the presence of SOD (from 54±6% to 84±10%, P<0.01) and that induced by 4 x 10^4 cells was significantly reduced in the presence of oxyHb (from 98±1% to 14±8%, P<0.0005). Preincubation of 4 x 10^4 cells with LMA significantly reduced their anti-aggregatory activity (from 98±1% to 24±4%, P<0.0005). This effect was reversed by concurrent incubation with L-arg (from 24±4% to 90±4%, P<0.0005) but not by D-arg (n=4, P<0.4). When rat mast cells (10^5 cells) were exposed alone to stirring (1000rpm) for various periods of time (1, 3 and 10 min), a significant time-dependent increase in their cGMP but not cAMP levels was observed. The increase in cGMP after 10 min of stirring was reduced in mast cells that had been pretreated with LMA for 60min (from 168±44 to 88±22 fmol/10^5 cells, n=7, P<0.05). This effect was reversed when LMA was co-incubated with L-arg (from 88±22 to 182±37 fmol/10^5 cells, n=7, P<0.05) but not with D-arg (n=7, P<0.45).

We have, therefore, clearly demonstrated that rat serosal mast cells release a factor which inhibits platelet aggregation in a manner indistinguishable from that described for NO. In addition, as the release of this NO-like factor is inhibited by LMA, it indicates that its release occurs through a mechanism similar to the one reported in endothelial cells and other white blood cells. The additional finding that exposure of mast cells to stirring increases their own levels of cGMP, an effect blocked by LMA, suggests that NO-like activity released from these cells not only infuences the behaviour of cells like platelets but also that its intra-cellular release may modulate mast cell reactivity to physiopathological stimuli.

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334P HUMAN PLACENTA: A NOVEL SOURCE OF MAST CELLS

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Human placenta was estimated to contain 0.72 ± 0.10 μg of histamine per g wet weight of tissue (4 placentas P: 2 normal delivery ND, 2 Caesarian section CS; n=17) following the tissue extraction procedure of Anton and Sayre (1969). After collagenase (1.5 mg/ml, 37ºC, 90 min) digestion of placental tissue, the calculated histamine content was not significantly different (0.56 ± 0.50 μg/g wet weight; 4 P: 2 ND, 2 CS; n=22). Overall, human placenta contains 0.63 ± 0.05 μg of histamine per g wet weight of tissue, values in agreement with others (Wicksell, 1949). There was no significant difference between the histamine content of placentas obtained from ND, compared with those collected at CS.

Mast cells were positively identified by virtue of morphology, staining characteristics and secretory activity. Enzymatic digestion of human placenta, using collagenase enzyme (1.5 mg/ml, 37ºC, 90 min) yielded 7.58 ± 0.44 x 10^5 mast cells/g wet weight of tissue (4 P: 2 ND, 2 CS; n=24), identified by their staining with toluidine blue, in a purity of 0.58 ± 0.04%. These cells stained with alcian blue, and were positive for chloro-acetate-esterase. The histamine content of a single mast cell was calculated to be 1.11 ± 0.09 pg/cell (4 P: 2 ND, 2CS; n=24). There was no significant difference in the number of mast cells or their histamine content between placentas collected after ND compared with those obtained at CS. Viability of dispersed cells, as assessed by trypan blue dye exclusion was >92%, and spontaneous histamine release was low (12.1 ± 1.5%). Linear regression analysis revealed a significant correlation (r=0.91) between the number of mast cells and the amount of histamine in aliquots of cell suspension derived from enzymatic dispersion.

Mast cells from human placenta released histamine in a concentration dependent manner, following challenge with compound 48/80, the calcium ionophore A23187 and a mixture of anti-human IgE and phosphatidylserine (10 μg/ml). Unlike other human tissues so far studied, with the exception of skin (Lowman *et al*, 1988), compound 48/80 was an effective liberator of histamine with an EC_{50} of 9.5 μg/ml and maximal release of 18.8 ± 1.3% at 30 μg/ml. Maximal histamine secretion with A23187 was not demonstrated, since the highest concentration of this agent (1 mg/ml) induced lysis of the cells. However, at 600 μg/ml A23187 output of histamine was 27.4 ± 1.2% of total. Anti-IgE was a potent secretagogue, and the concentration effect curve was still rising (30% histamine output) with the maximal concentration of anti-IgE available (1/2 dilution).

Thus, the placenta represents a novel source of human mast cells, present in sufficient numbers for experimental use and easily dispersed by a simple collagenase digestion technique. Dispersed mast cells are morphologically intact, exhibit a low spontaneous output of histamine and are responsive to several commonly used secretagogues.

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335P MPTP-INDUCED ATP DEPLETION IN SYNAPTOSOMES IS INHIBITED BY SELEGILINE BUT NOT MAZINDOL

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1-Methyl-4-phenylpyridinium species (MPP^+) inhibits complex I of the mitochondrial electron transport chain (Ramsay et al, 1986). This may lead to ATP depletion and ultimately cell death, but depletion of ATP by MPTP or MPP^+ has not been observed in in vitro synaptosomal preparations of neuronal origin. Currently the effects of MPTP and MPP^+ on ATP levels in mouse brain synaptosomes, have been investigated.

Mouse brain synaptosomes were incubated at 37°C in the presence or abs' ence of mazindol 10μM or selegiline 20μM and MPTP 1mM or MPP^+ (0.1 or 1mM). ATP was measured by chemiluminescence. MPP^+ accumulation was measured in synaptosomes preincubated for 10 minutes at 37°C with or without mazindol 10μM, and 50μM [^{3}H]-MPP^+.

Table 1. ATP levels in synaptosomes after MPTP or MPP^+

	ATP (% of Initial values)				
	15 mins	60 mins		15 mins	60 mins
Control	90.2 ± 1.5	81.4 ± 2.8	Selegiline 20μM + MPTP 1mM	93.5 ± 4.3	81.5 ± 5.3
MPTP 1mM	83.8 ± 1.0*	61.1 ± 1.7*	Selegiline 20μM + MPP^+ 1mM	62.0 ± 3.4*	44.0 ± 4.2*
MPP^+ 1mM	67.0 ± 1.5*	49.4 ± 4.4*	Mazindol 10μM	88.3 ± 1.3	79.7 ± 1.6
MPP^+ 0.1mM	83.7 ± 2.2*	63.6 ± 1.8*	Mazindol 10μM + MPP^+ 0.1mM	84.8 ± 2.0*	65.2 ± 1.9*
Selegiline 20μM	93.1 ± 5.6	80.2 ± 3.8	Mazindol 10μM + MPTP 1mM	81.1 ± 2.5*	62.9 ± 4.1*

Results were mean ± SEM, n= 3-6 experiments. Comparisons were made to control values by 2-way ANOVA *$p<0.05$.

MPTP and MPP^+ depleted ATP in synaptosomes. Selegiline prevented the ATP depletion produced by MPTP but not MPP^+. Mazindol did not attenuate the ATP depletion produced by MPTP or MPP^+. In addition mazindol did not inhibit MPP^+ accumulation at 30 minutes (MPP^+ 50μM, 0.4 ± 0.02 and mazindol 10μM + MPP^+ 50μM, 0.3 ± 0.04 nmoles/mg protein).

MPTP and MPP^+ decreased ATP in synaptosomes, but this decrease may be non-specific for catecholaminergic neurons and maybe the result of accumulation of MPP^+ by a process not involving the catecholamine reuptake carrier.

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336P BEHAVIOURAL EFFECTS OF ROLIPRAM IN MPTP-TREATED COMMON MARMOSETS

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Rolipram is a selective inhibitor of one isoenzyme of brain phosphodiesterase (PDE) producing an increase in cyclic AMP availability (1). PDE inhibitors of this type, including rolipram, induce a characteristic syndrome of head twitches, grooming, forepaw shaking and decreased locomotion in normal rats but not mice (2). However, following monoamine depletion by reserpine plus α-methyl-p-tyrosine, rolipram and similar compounds induce an increase in locomotor activity in both species. These data might suggest a role for PDE inhibitors, such as rolipram, in the treatment of Parkinson's disease. We now report on the effects of rolipram on the behaviour of MPTP-treated common marmosets.

Common marmosets were employed which had been treated with 10-12 mg/kg s.c. of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) 18 months prior to the experiment. These animals showed a reduced basal locomotor activity when measured automatically using photocell cages but otherwise were not obviously different from normal controls. Rolipram (1.25 and 2.5 mg/kg ip) or vehicle (10% w/v Tween 80/0.9% saline) was administered ip in a latin square design with one week's interval between experiments. Locomotor activity was recorded for 2 hours while other behavioural events were observed through a one-way mirror.

Administration of roliparam (1.25 or 2.5 mg/kg ip) to MPTP-treated common marmosets induced salivation, head twitching and excessive grooming. The syndrome was rapid in onset appearing after 10 min and lasted for approximately 60 min. Locomotor activity was not increased after administration of 1.25 mg/kg rolipram but there was a tendency for an increase at 2.5 mg/kg although this did not reach statistical significance.

Table 1: Behavioural effects of rolipram in MPTP-treated marmosets

	Mean counts per 120 min (SEM, N = 3)		
	Vehicle	1.25	2.5 mg/kg ip
Head twitches	0	13 (6.1)	8 (2.5)
Grooming	0	4.3 (2.6)	4.6 (2.3)
Locomotor activity	769 (484)	595 (255)	1127 (384)

Rolipram, when administered to MPTP-treated common marmosets, elicits qualitatively the same syndrome seen in normal rodents. At the dose used no significant antiparkinsonian activity was observed but it remains to be determined whether the drug would synergise with dopamine agonist treatments.

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337P MODULATION OF CORTICOTROPHIN (ACTH) RELEASE *IN VITRO* BY METHYLXANTHINES AND ADENOSINE ANALOGUES

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Methylxanthines influence the secretion of a number of pituitary hormones including ACTH. It is not yet known whether they act directly on the adenohypophysis or whether they influence the release of the hypothalamic hormones which regulate anterior pituitary function. Nor is it apparent whether they involve inhibition of phosphodiesterase (PD), and thus elevation of intracellular cAMP, or blockade of adenosine receptors. In these experiments the effects of two methylxanthines, denbufylline [which has been shown to be more potent in inhibiting a cAMP specific PD than in blocking A1-adenosine receptors (Nicholson et al. 1989)] and caffeine, on the secretion of ACTH by rat pituitary segments in vitro were examined in the presence and absence of hypothalamic extracts (HE) and a variety of adenosine receptor agonists. Pituitary tissue was collected post mortem from adult male Sprague-Dawley rats and incubated (Buckingham & Hodges, 1977) using a 60 min. contact time with each secretagogue. The ACTH released into the medium was determined by radioimmunoassay. Hypothalamic extracts (0.05-0.4 HE/ml) caused significant ($P<0.01$) concentration dependent increases in ACTH release producing, at the highest concentration, a seven-fold increase in peptide release. Their effects were mimicked by the selective A1-adenosine receptor agonists, N6-cyclohexyladenosine (0.1-10nM) and N6-(phenylisopropyl)-adenosine (0.1-10nM), each of which produced pronounced ($P<0.01$) concentration dependent increases in ACTH release. Two other adenosine analogues, 5-N-ethylcarboxamidoadenosine (1-100nM) and 5-[N-cyclopropyl]- carboxamidoadenosine (1-100nM), reputed to show some degree of selectivity for A2 receptors, were without effect. In high concentrations, denbufylline (0.01-1mM) and caffeine (8-16mM) precipitated small, but significant ($P<0.05$) increases in ACTH release. In lower concentrations (0.1-1μM and 0.05-4mM respectively), both methylxanthines potentiated markedly ($P<0.05$), the secretory responses to a submaximal concentration of hypothalamic extract (0.1 HE/ml). In addition, denbufylline (0.1μM-1mM) effectively blocked ($P<0.01$) the secretory responses to both A1-adenosine receptor agonists. When these experiments were repeated using acutely dispersed pituitary cells maintained under static or perifusion conditions, similar profiles of data were obtained. The results show clearly that methylxanthines act directly on the pituitary gland to influence ACTH secretion and provide preliminary novel evidence for a role for adenosine receptors in the control of this pituitary hormone. The finding that high concentrations of caffeine and denbufylline are required to elicit ACTH release directly but that lower concentrations effectively potentiate the cAMP-mediated corticotrophic response to hypothalamic extracts is in accord with an action on phosphodiesterase. Denbufylline also appears to have the capacity to block pituitary A1-adenosine receptors.

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338P EVIDENCE THAT VASOPRESSIN CONTRIBUTES TO THE STRESS-INDUCED SUPPRESSION OF GONADOTROPHIN RELEASE

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Stress not only activates the pituitary-adrenocortical axis but causes a depression of gonadotrophin secretion. The former response is initiated by two hypothalamic hormones, the 41-amino acid corticotrophin releasing factor CRF-41 and arginine vasopressin (AVP), which act synergistically at the pituitary level to promote ACTH release. CRF-41 also inhibits the release of gonadotrophin releasing hormone by the hypothalamus (Gambacciani et al., 1986). Reports that AVP is co-localized with CRF-41 in the parvocellular neurones of the paraventricular nucleus (Whitnall, 1988) raise the possibility that this neuropeptide may also be important in this respect. Accordingly, we have examined, using established biological and radioimmunological assay methods, the effect of stress on the secretion of ACTH, corticosterone and luteinizing hormone (LH) in Brattleboro rats (in which a single base deletion in the pre-provasopressin gene prevents the synthesis of AVP) and in normal controls of the parent strain (Long Evans). In the Long Evans rats stress (0.6mg/100g histamine, i.p.) initiated, within 5 and 20 min respectively significant ($P<0.01$, Student's 't' test) increases in the plasma ACTH (0 min = 18.2 ± 2.0mU/l; 5 min = 36.2 ± 11.0mU/l, n = 8) and corticosterone (0 min = 19.1 ± 5.9μg/l; 20 min = 48.2 ± 8.1μg/l, n = 8) concentrations. It also caused a reduction in the serum LH concentration which was maximal at 5 min (pre-stress = 0.84 ± 0.1μg/l; 5 min = 0.54 ± 0.05μg/l, $P<0.05$, Student's 't' test, n = 8). By contrast, in the vasopressin deficient Brattleboro rats, stress had no effect on the serum LH concentration and produced only modest increases in pituitary adrenocortical activity compared with those in Long Evans controls. Thus the plasma ACTH and serum corticosterone concentrations attained within 5 and 20 min respectively were 23.4 ± 6.7mU/l and 23.0 ± 6.4mU/l (n = 5). Pretreatment of both Long Evans and Brattleboro rats with dexamethasone (20μg/100g i.p., 10.0 a.m. for 3 days) effectively abolished the pituitary-adrenal response to stress. The steroid treatment also prevented the stress-induced suppression of LH in the Long Evans rats; indeed, unlike the vehicle-treated controls, these animals exhibited, within 5 min, a rise in serum LH concentration when exposed to stress (pre-stress = 0.6 ± 0.1μg/l; 5 min = 1.4 ± 0.1μg/l, $P<0.01$, Student's 't' test, n = 8). Stress did not affect the serum LH concentrations in steroid-treated Brattleboro rats. The results confirm previous reports that AVP is required for the full expression of the pituitary-adrenocortical response stress. They also provide novel evidence which suggests that AVP release in stress contributes to the impairment of gonadotrophin secretion.

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339P ETHANOL WITHDRAWAL: A ROLE FOR CORTICOTROPHIN-RELEASING FACTOR (CRF)

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Intracerebroventricular (i.c.v.) administration of corticotropin-releasing factor (CRF) has anxiogenic-like effects in animal tests of anxiety (Britton et. al. 1986; Dunn & File, 1987). Similar effects occur in rats withdrawn from chronic ethanol (File et. al., 1989). This study examined whether increased activity of endogenous CRF mediates the behavioural effects of ethanol withdrawal in the rat.

In Experiment 1 naive Wistar rats were tested in the elevated plus-maze (Pellow et. al., 1985) after CRF (0.1 & 0.5 ug i.c.v.) or the CRF-receptor antagonist, alpha-helical CRF (9-41) (5 & 25 ug i.c.v.). CRF (0.5 ug) reduced the % of time spent on the open arms indicating anxiogenic-like activity (saline=31±6.5,n=9;CRF=10±3.4,n=9;t=2.92, $p<0.05$). Alpha-helical CRF (5 & 25 ug) had no effect (saline=18±5;5ug=20±9;25ug=16±5).

In Experiment 2 rats were maintained for 2-3 weeks on a nutritionally balanced liquid-diet containing ethanol (8.5-11.5% v/v) or sucrose. 8 h after withdrawal from ethanol rats spent a reduced % of time on the open arms compared with controls (control=57±8.4; withdrawal=28±8.1 ANOVA $F(1,27)=4.5,p<0.05$). Alpha-helical CRF (5 & 25 ug i.c.v.) reversed the anxiogenic-like effects of ethanol withdrawal (withdrawal=28±8.1;5ug=57.3 ±10.8;25ug=48±8.1 planned-ANOVA $F(1,38)=4.45,p<0.05$). Other behavioural signs of ethanol withdrawal (tremor, tail-stiffness, ventromedial distal flexion) were unaffected by alpha-helical CRF. Blood alcohol levels on withdrawal were similar in all 3 groups (withdrawal=211±8;5ug=197±11;25ug=212±9 mg/dl).

These results support the hypothesis that the enhanced anxiety during ethanol withdrawal may be due to increased activity of endogenous CRF.

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340P BACLOFEN REVERSES THE SIGNS OF ETHANOL WITHDRAWAL IN RATS

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Chronic ethanol treatment reduces brain GABA concentrations & binding sites (Ticku and Burch, 1980; Hunt, 1983) and $GABA_A$ receptor agonists suppress some signs of the ethanol withdrawal (EW) syndrome (Frye et al.,1983). We studied the effect of the $GABA_B$ receptor agonist, baclofen (BAC) on the increased anxiety and tremor seen during ethanol withdrawal. Male hooded Lister rats were fed a 20% w/v liquid chocolate Complan diet to which increasing concentrations of absolute ethanol were added to reach 10% which was maintained for a further four weeks (final intake of ethanol 14.5±0.9 g/kg/day). 7.5 h after ethanol withdrawal, rats were given BAC (1.25-5.0 mg/kg i.p.) or vehicle and 30 min later tested in the social interaction and elevated plus-maze tests of anxiety and scored for the incidence of tremor. Withdrawal from ethanol resulted in an increase in anxiety, shown by reductions in time spent in social interaction [$P<0.01$] & in % entries on the open arms of the plus-maze [$P<0.05$]. There was also increased aggression [$P<0.001$] and tremor [$P<0.001$] BAC (5 mg/kg) reduced locomotor activity in both control and EW rats. BAC (1.25-2.5 mg/kg) did not affect behaviour of control animals, but in EW rats increased the time spent in social interaction ($P<0.05$) and % number of entries on the open arms of plus maze [$P<0.05$] indicating a reversal of the anxiogenic response during EW. BAC (1.25-5.0 mg/kg) dose dependently suppressed aggression [$P<0.05$] and tremor [$P<0.05$] in EW rats. The results of the present study indicate that BAC might be useful in the treatment of some signs of EW.

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341P RELATIVE AGONIST POTENCIES OF METHYLENE SUBSTITUTED ATP ANALOGUES FOR P_{2X} PURINOCEPTORS IN THE RABBIT EAR ARTERY

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Stimulation of P_{2X}-purinoceptors results in contraction of vascular (e.g rabbit ear artery, Kennedy and Burnstock, 1985) and visceral smooth muscle (e.g guinea-pig bladder, Cusack and Hourani, 1984). Classification of this receptor sub-type has, to date, been based on the relative agonist potencies of a limited series of ATP analogues, especially those with methylene substitutions in the phosphate chain. L-βγmethylene ATP has been reported to be a stable selective P_{2X}-agonist in the bladder and the most potent agent of this type tested in this tissue (Cusack and Hourani, 1984, Hourani et al., 1986). We have examined the potency of L-βγmethylene ATP to contract the rabbit ear artery and compared it with D-αβmethylene ATP, D-βγmethylene ATP and ATP.

Central ear arteries from male Nz. White rabbits (2.5-3kg) were denuded of endothelium, cut into rings and suspended under 1g resting tension in Krebs solution at 37°C gassed with 95%O_2 / 5%CO_2. All experiments were performed in the presence of indomethacin (2.8×10^{-6}M) and the selective P_1-purinoceptor antagonist 8-sulphophenyltheophylline (3×10^{-4}M). In each tissue cumulative agonist-concentration effect (E/[A]) curves were constructed to D-αβmethylene ATP as standard and one of the ATP analogues listed above. The mechanism of the contractile responses produced was assessed by repeating the E/[A] curve after 15 min exposure to a maximal concentration of D-αβmethylene ATP (3×10^{-5}M) which selectively desensitises P_{2X}-receptors.

All compounds were full agonists as defined by the maximum response to D-αβmethylene ATP. Relative potency order was as follows, pA_{50} values (mean ± s.e, n=4-6) are shown in brackets: D-αβmethylene ATP (6.47 ± 0.04) > L-βγmethylene ATP (5.52 ± 0.04) > D-βγmethylene ATP (4.37 ± 0.12) > ATP (3.14 ± 0.14). Contractions to all agonists, except ATP, were effectively abolished by desensitisation with D-αβmethylene ATP. ATP produced some residual contractions.

Hence, at P_{2X}-receptors in the rabbit ear artery, L-βγmethylene ATP is considerably more potent than its D-isomer but 10-fold less potent than D-αβmethylene ATP. This agonist potency order is similar to that reported for rat portal vein (Reilly and Burnstock, 1987) but differs from that described for P_{2X}-receptors in the guinea-pig bladder where L-βγmethylene ATP is the most potent agent and D-βγmethylene ATP and D-αβmethylene ATP have similar activity (Cusack and Hourani, 1984, Welford et al., 1987). In view of these differences, and the apparent variation in the P_{2X}-antagonist potency of suramin between tissues (Wood et al., 1990), further quantitative studies of the P_{2X}-purinoceptors mediating spasmogenic effects in vascular and visceral smooth muscle preparations are warranted.

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342P SURAMIN IS A SLOWLY EQUILIBRATING BUT COMPETITIVE ANTAGONIST AT P_{2X}-PURINOCEPTORS IN THE RABBIT EAR ARTERY

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The classification of P_2-purinoceptors is limited by the lack of selective competitive antagonists. Suramin has been reported (Dunn & Blakeley, 1988) to be a P_{2X}-receptor antagonist in the mouse vas deferens, although it is devoid of effect in the guinea-pig urinary bladder (Hourani & Chown, 1989). However, no data have been presented that establish whether suramin's antagonistic action is genuinely competitive. This study describes an attempt to provide such data.

Experiments were conducted using isolated rings of endothelially-denuded ear artery from NZW-rabbits (O'Connor et al.,1990). αβmethylene ATP concentration-effect curves were constructed cumulatively in a paired curve design in the absence and presence of increasing concentrations of suramin incubated for 45 min. The slope of the resulting Schild plot was greater than unity (1.50±0.08, 18 df). An explanation for this result was insufficient antagonist equilibration time (Kenakin, 1980). This was confirmed by examining the effects of a low (3×10^{-5}M) and a high (10^{-3}M) concentration of suramin using a short (15min) and a long (3 hr) incubation period. The slopes of the resulting Schild plots were 1.66 ± 0.36 (10 df) (15min incubation) and 1.06 ± 0.13 (11 df) (3hr incubation).

However, following 3hr incubation with 10^{-3}M suramin a depression of the maximum response to αβmethylene ATP occurred. In order to attempt to minimise this effect, we investigated the kinetics of suramin and determined the minimum incubation times required to achieve an effective equilibrium with each concentration of the antagonist. Suramin (3×10^{-5}M) was incubated for 30,60,120,180 and 240 min and dose ratios (r) calculated at each time point. Theoretically the fractional occupancy of an antagonist is given by (r-1)/r (Paton, 1961). Thus a plot of (r-1)/r versus time allowed us to calculate the onset (k_1) and offset (k_{-1}) rate constants: 306 ± 45 min^{-1} M^{-1} and 4.40 ± 1.52 x $10^{-3}$$min^{-1}$ respectively. k_{-1}/k_1, the equilibrium dissociation constant, was 1.44×10^{-5}M, corresponding to a pK_B of 4.84. Using these estimates we calculated the minimum time for different concentrations of suramin to achieve 95% occupancy. Under these conditions suramin fulfilled the criteria for simple competition, parallel curve displacements and a unit Schild plot slope (1.00 ± 0.09, 23 df). The pK_B estimate was 4.79 +/- 0.05, 24 df. Suramin (10^{-3}M) incubated for 15 min (which allows 95% equilibration at P_{2X}-receptors) had no effect on KCl concentration-effect curves and produced only slight rightward displacements of phenylephrine (ΔpA_{50} ± s.e, 0.22 ± 0.12, 10 df) and histamine (0.36 ± 0.06, 8 df) curves.

These studies provide evidence that suramin is a slowly equilibrating but competitive P_{2X}-receptor antagonist in the rabbit ear artery. As such, suramin may prove to be a useful tool in the classification of purinoceptors.

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343P P_2 PURINOCEPTOR-LINKED STIMULATION OF LABELLED PHOSPHATIDIC ACID PRODUCTION IN BOVINE AORTA ENDOTHELIAL CELLS.

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ATP has previously been shown to act on P_2-purinergic receptors on endothelial cells of the thoracic aorta to stimulate phospholipase C (1) and of the pulmonary artery to stimulate phospholipase D (2). Here we report on the characteristics of phosphatidic acid (PA) production following stimulation of P_2 receptors on aortic endothelial cells. PA may arise directly by P_2 receptor stimulation of phospholipase D, but it may also arise indirectly by receptor linked activation of phospholipase C and subsequent phosphorylation of diacylglycerol.

We used a bovine thoracic aorta derived endothelial cell line (AG 4762) grown as a monolayer on 24-well plates prelabelled with 0.25 MBq/ml [^{32}P]orthophosphate for 2.5h. After stimulation of cells, usually for 1 min at room temperature, lipids were extracted into chloroform, and run on silica gel thin layer plates. The PA spot was scraped, eluted and counted.

The relatively P_{2Y} selective agonist 2-methylthio ATP (2MeSATP) stimulated the production of [^{32}P]PA over the concentration range 0.1μM to 30μM, with an EC_{50} of 1-2μM, with the maximal response being 4-10 times basal. The time course of the response was a rapid rise over 1 min with a slower and more sustained rise over 10 min. Over this concentration range the relatively P_{2X} selective agonist β-γ-methylene ATP failed to stimulate the accumulation of counts in PA.

The protein kinase C stimulating phorbol ester, tetradecanoyl phorbol acetate (TPA), had little or no effect on basal production of [^{32}P]PA. However, 10 min preincubation with 100nM TPA did inhibit the 2MeSATP stimulated production of counts in [^{32}P]PA (Table 1). We found a similar inhibition by TPA of 2MeSATP stimulated [^{3}H]inositol phosphate in cells prelabelled with [^{3}H]inositol.

Table 1 - Effect of phorbol ester

	Control	TPA (100 nM)
Basal	516 ± 39	514 ± 44
2MeSATP	1705 ± 97	933 ± 168

Figures are c.p.m. [^{32}P]PA, n=3, ± s.e. mean

These results show that PA formation in thoracic aorta endothelial cells may be enhanced by stimulation of P_{2Y} receptors. This response may be downstream of phospholipase C stimulation. It is not however, due to stimulation of protein kinase C by diacylglycerol.

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344P CHARACTERIZATION OF GUANINE NUCLEOTIDE-SENSITIVE BRADYKININ (BK) BINDING SITES IN BOVINE AORTIC ENDOTHELIAL CELLS

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To investigate the underlying mechanisms of BK-induced production of autacoids, BK-binding sites and their sensitivity to guanine nucleotides were studied in endothelial cells.
Primary, second passaged bovine aortic endothelial cells were grown to confluence (see Schini et al., 1988), washed, homogenized and centrifuged at 100,000xg for 60 min at 4°C. The 100,000xg pellet was resuspended in 25 mM TES, 1 mM 1,10-phenanthroline, pH 6.8 and used as membrane preparation. The binding incubation mixture consisted of 0.2 % BSA, 14 μg/ml bacitracin, 10 μM captopril, 100 μM DTT, 1 mM 1,10-phenanthroline, 25 mM TES, pH 6.8, various concentrations of [^{3}H]BK, with or without 1 μM unlabelled BK, and 0.8 mg/ml protein of membrane preparation in a final volume of 500 μl. The binding assay was performed at 25°C for 90 min. At the end of the incubation period the solutions were filtered under reduced pressure through Whatman GF/B glass fibre filters. Specific [^{3}H]BK binding (100 pM and 500 pM) to endothelial cell membranes was linearly related to tissue concentrations between 0.2 - 1.6 mg/ml of protein. All subsequent experiments were carried out at 0.8 mg/ml protein. Kinetic experiments showed that specific [^{3}H]BK binding (100 pM and 500 pM) reached equilibrium within 40 min at 25°C. Specific [^{3}H]BK binding using 40-1000 pM concentrations of [^{3}H]BK was saturable and Scatchard analysis indicated the presence of a high affinity site over this concentration range with a K_D value of 152±28 pM (s.e.mean) and a B_{max} of 4.6±0.6 fmol.mg^{-1} (n=9). Unlabelled BK displaced specific [^{3}H]BK binding (700 pM) with an IC_{50} of 554±88 pM and a slope factor of 1.06±0.09 (n=5) whereas other vasoactive peptides such as angiotensin II, AVP, endothelin-1, and ANP (30 nM - 1 μM) did not alter the binding of [^{3}H]BK. The B_2-agonist kallidin and the B_2-antagonists D-Arg^{0}[Hyp^{3}, Leu^{5,8}, Gly^{6}, D-Phe^{7}]BK, D-Arg^{0}[Hyp^{3}, D-Phe^{7}]BK, [D-Phe^{7}]BK, and [Thi^{5,8}, D-Phe^{7}]BK inhibited specific [^{3}H]BK binding (700 pM) with respective IC_{50} values of 395±85 pM (n=4), 1.22±0.24 nM (n=4), 3.50±0.50 nM (n=5), 9.81±2.20 nM (n=5), and 26.9±6.3 nM (n=5), and respective slope factors of 1.04±0.05, 1.17±0.09, 0.82±0.06, 0.91±0.10, and 0.94±0.08. The B_1-antagonist des-Arg^{9}[Leu^{8}]BK had no effect up to 1 μM. GTP, GTPγS, GDP and GDPβS inhibited specific [^{3}H]BK binding (100 pM and 500 pM) almost fully at 100 μM whereas 5'-GMP, guanosine, cGMP, ATP, ADP, 5'-AMP and adenosine had no effect at 100 μM. Specific [^{3}H]BK binding (100 pM and 500 pM) was inhibited by GTP and GDP with an IC_{50} of 1-3 μM. GTP and GDP at 3 μM decreased the B_{max} value by 70% and 40% respectively.
This study demonstrates the existence of specific high affinity B_2-BK binding sites selectively inhibited by the guanine nucleotides GTP and GDP suggesting an interaction of endothelial cell B_2-BK binding sites with a G protein.

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345P *IN VITRO* AUTORADIOGRAPHY OF [^{3}H]-RX821002 IN THE RAT CNS, A NEW LIGAND FOR IDENTIFYING α_2-ADRENOCEPTORS

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RX821002 (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazololine) is an analogue of the selective α2-adrenergic antagonist, idazoxan, that has improved potency and selectivity in in vitro studies (Stillings et al.,1985). Recently [3H]RX821002 has become available and binding studies indicate selectivity for α2a-adrenoceptors in HT29 adenocarcinoma cell line (Langin et al.,1989). In human frontal cortex RX821002 has comparable affinity with rauwolscine at α2-adrenoceptors but low affinity for non-adrenoceptor idazoxan binding sites (Vauquelin et al.,1990). We have now evaluated this ligand as a tool for autoradiography of α2-adrenoceptors.
Male Sprague-Dawley rats (220g) were given an intracardiac perfusion of phosphate buffered saline under pentobarbitone anaesthesia (40 mg/kg i.p.). Brains were rapidly frozen, cut on a cryostat and thaw mounted onto glass slides. For binding, sections were prewashed in tris-HCl buffer (50mM,pH 7.4) containing 1 mM Mg^{2+} prior to incubation in 5 nM [3H]RX821002 with or without unlabelled drugs for 20 min at 22°C. Sections were rinsed twice in fresh icecold buffer, rapidly dried, apposed to tritium sensitive film for 6 weeks with appropiate standards to facilitate image analysis and quantitation of labelled sites.
The autoradiographic distribution of [3H]RX821002 binding sites closely resembles that reported for the α2-agonist, [3H]para-aminoclonidine (Unnerstall et al.,1984) and [3H]idazoxan but differs from that of [3H]rauwolscine (Boyajian et al.,1987). Binding was attenuated in the presence of unlabelled drugs, as shown in the table below (fmol/mg wet tissue, means ± s.e.m., n=3 rats).

	Frontal cortex	Anterior olfactory nucleus	Lateral septal nuclei	Inferior colliculus	Posterior hypothalamic area	Nucleus tractus solitarus
Total	145.30±13.3	356.10±22.3	354.30±8.4	179.30±12.9	305.00±18.0	181.70±20.1
Idazoxan 1uM	0.39±0.19	0.94±0.16	0.60±0.17	0.79±0.71	0.94±0.05	1.51±0.60
RX821002 1uM	1.40±1.34	1.34±1.21	1.49±1.14	2.23±1.91	2.29±1.30	3.47±2.04
Yohimbine 5uM	29.60±4.35	91.20±14.9	68.60±34.4	48.50±9.07	89.50±7.64	38.2±8.52

[3H]RX821002 appears to be a useful ligand for autoradiography, having high affinity for α2-adrenoceptors and a low degree of non-specific binding. The reasons for it's different distribution compared with [3H]rauwolscine binding, and the incomplete inhibition by yohimbine require further investigation.

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346P RADIOLIGAND BINDING TO PRESYNAPTIC α_2-ADRENOCEPTORS IN RAT CORTEX REVEALED BY SHORT-TERM DSP-4 LESIONING

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Attempts to demonstrate presynaptic α_2-adrenoceptors by radioligand binding following lesion of noradrenergic neurons have been unsuccessful. In fact most studies have reported increased binding when assayed 10-42 days after lesioning (Bylund and U'Prichard 1983) suggesting that denervation supersensitivity occurs at postsynaptic receptors. In this study we have examined the effect of the noradrenergic neurotoxin DSP-4 on α_2-adrenoceptors in rat cerebral cortex 3 and 14 days after treatment, using the full agonist [^{3}H]UK14304 (5-bromo-6-[2-imidazolin-2-yl amino] quinoxaline) and the antagonist [^{3}H]rauwolscine.
Male Wistar rats (250-350 g) were pretreated with zimeldine (10 mg/kg i.p.) then 30 minutes later received either DSP-4 (100 mg/kg i.p.) or 0.5 ml saline. Animals were killed either 3 or 14 days after treatment and the brain immediately removed. Specific binding of [^{3}H]rauwolscine and [^{3}H]UK14304 binding was defined by 10^{-6} M phentolamine and 10^{-4} guanylylimidodiphosphate respectively.
Table 1: α_2-Adrenoceptor binding in rat cerebral cortex following DSP-4 treatment

	[^{3}H]Rauwolscine				[^{3}H]UK14304			
	Control		DSP-4		Control		DSP-4	
	Bmax	Kd	Bmax	Kd	Bmax	Kd	Bmax	Kd
3 DAYS (n=5)	183 ± 13	3.60 ± 0.25	151 ± 11*	3.60 ± 0.4	102 ± 8	1.30 ± 0.17	77 ± 5*	0.97 ± 0.03*
14 DAYS (n=4)	180 ± 10	3.15 ± 0.23	168 ± 12	3.03 ± 0.4	118 ± 10	1.08 ± 0.16	131 ± 9*	1.00 ± 0.12

Binding capacity (fmol/mg protein) and affinity (nM) of [^{3}H]rauwolscine and [^{3}H]UK14304 in rat cerebral cortex following treatment with DSP-4 or saline. * $P<0.05$ vs corresponding control value (paired t-test).

As shown in Table 1, 3 days after treatment with DSP-4 the number of α_2-adrenoceptors was significantly decreased for both agonist (25%) and antagonist (18%) binding. The affinity of the agonist radioligand also increased by 30%, similar to the findings of Dooley et al. (1983). The loss in number of receptors was no longer apparent 14 days after treatment, at which time the antagonist binding was not significantly different from control value, whereas the agonist binding was significantly increased (11%).
We therefore propose that 3 days after treatment with DSP-4 both agonist and antagonist binding capacities are decreased due to the loss of presynaptic α_2-adrenoceptors following destruction of the noradrenergic nerve terminals. After 14 days the number of receptors returns to control values due to the effect of denervation supersensitivity on postsynaptic α_2-adrenoceptors and the proportion of receptors adopting the high-affinity agonist state is increased after DSP-4 compared to controls.

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347P USE OF A ROTATIONAL MODEL TO DEMONSTRATE THE EXISTENCE OF NEUROKININ NK-2 RECEPTORS IN THE RAT SUBSTANTIA NIGRA

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The substantia nigra (SN) is known to contain the highest concentration of the neurokinins (NK), substance P (SP) and NKA, in the brain (Arai & Emson 1986). Correspondingly high numbers of NK receptors have not been located within the SN and the nature of the NK receptors in this area remains unclear. Unilateral activation of the nigro-striatal dopamine (DA) pathway is known to induce contralateral rotational activity (Anden et al 1966). This DA pathway is known to be under an excitatory NK tone (Cheramy et al, 1977); and unilateral injection of SP into the SN can elicit contralateral rotations (Olpe & Koella, 1977). We have investigated, using selective NK receptor agonists and a recently described NK-2 antagonist, cyclo (GlnTrpPheGlyLeuMet) (L-659,877; Maguire et al, 1989), the NK receptors mediating this rotational response.

Chronic bilateral guide cannulae were implanted 1 mm above the SN in male rats (Glaxo; 250-300g). One week following surgery, rats received unilateral drug infusions (1 µl/side over a 1 min period) into the SN. Both contra- and ipsilateral rotations were recorded for 90 mins in hemispherical bowls using an automated system. Microinfusion of a selective NK-2 agonist, δ-Ava[D-Pro^9]SP(7-11) (Ireland et al, 1988) into the SN induced significant dose-related increases in the number of contralateral rotations (vehicle 16±2; GR51667 0.7 µg 69±9, 3.5 µg 93±8 rotations 60min^{-1}). The response to 3.5 µg of the agonist was antagonised by co-administration with the NK-2 antagonist L-659,877 at a dose of 4.0 µg. No rotational activity was observed following intra-SN microinfusion of the NK-1 selective agonists, SP-methylester (1-15 µg) or δ-Ava [L-Pro^9, N-Me-Leu^{10}] SP(7-11) (GR73632, Hagan et al, 1989) (0.77-7.66 µg) or the NK-3 agonist, senktide (0.1-1 µg), confirming the selectivity of the NK-2-mediated response. These data demonstrate the existence of functionally active NK-2 receptors in the rat SN. Furthermore, the results show that the rat rotational model can be useful for the identification of functional receptor sub-types in vivo.

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348P CCK_B RECEPTOR ACTIVATION CAUSES AN INCREASE IN ENDOGENOUS AMINO ACID RELEASE FROM THE STRIATUM AND VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS *IN VITRO*

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Autoradiographical studies have indicated a wide distribution of cholecystokinin (CCK) receptors throughout the central nervous system. In the ventromedial nucleus of the hypothalamus (VMH) a high density of ^{125}I-BH-CCK-8S binding sites (Day et al, 1986) has correlated with electrophysiological studies which have demonstrated an excitatory action of CCK mediated through the CCK_B receptor (Boden and Hill, 1988). In the striatum a cortico-striatal CCK pathway has been identified (Meyer et al, 1982) and CCK receptors have been located on neurones whose cell bodies originate within the striatum (Beresford et al, 1987).

In the present study, the effect of cholecystokinin octapeptide (CCK-8S) on amino acid release from slices of the striatum and the VMH was examined. Amino-acid release was measured by HPLC with fluorimetric detection. CCK-8S was found to increase basal, but not stimulated, release of aspartate, glycine and GABA. A dose-dependent significant increase in the levels of each amino acid was found with 500 and 1000nM CCK-8S. The CCK_B selective antagonist L-365,260 (Lotti and Chang, 1989) significantly antagonised CCK-8S evoked amino-acid release at a concentration of 10^{-7}M.

In addition, the CCK_A receptor antagonist L-364,718 (Chang et al, 1988) was ineffective at 10^{-8}M though antagonism of the CCK-8S effect was seen at the non-selective concentration of 10^{-5}M. Experiments to determine the calcium dependency of the CCK-8S evoked amino-acid release demonstrated that removal of extracellular calcium and the addition of EGTA did not significantly reduce the CCK-8S effect. Furthermore, the addition of 100nM ω-conotoxin , the calcium channel blocker, did not prevent the increased amino-acid release evoked by CCK-8S in normal aCSF.

These data suggest that CCK_B receptor activation in the VMH and striatum results in an increase in the endogenous release of aspartate, glycine and GABA. However, this process is not dependent on the presence of extracellular calcium.

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349P CHOLECYSTOKININ EXCITES DORSAL RAPHÉ NEURONES VIA A CCK_A RECEPTOR

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A population of neurones in the dorsal raphe has been shown to be hyperpolarised by 5-HT which increased the permeability to potassium ions (Williams, Colmers and Pan 1988) and excited by phenylephrine which blocked I_A (Aghajanian 1985). In the present study the effects of sulphated cholecystokinin octapeptide (CCK) were examined on neurones in an in vitro preparation of the raphe which responded to 5-HT and phenylephrine.

Intra and extracellular recordings were made using conventional techniques from dorsal raphe neurones in the slice preparation made from rat pons-mesencephalon. Coronal slices (350u) were cut and a single slice was placed in a tissue bath through which flowed physiological artificial cerebrospinal fluid (ACSF) (2ml/min) at 35°C as previously described (Williams, Colmers and Pan). Drugs dissolved in ACSF were applied by superfusion. L-364,718 and L-365,260 were synthesised in house.

In agreement with previous studies (Williams, Colmers and Pan 1988), two populations of neurones were observed in the raphe. Only the results from neurones which responded to 5-HT and phenylephrine are reported here. Fifteen neurones did not respond to CCK. Of the 41 neurones which responded to CCK (10-100nM) 32 were excited and 9 were inhibited. A sixty second application of CCK produced a response which lasted for between two and five minutes. Pentagastrin (1 to 10uM) had no effect on CCK sensitive neurones (n=3). Bath application of the CCK_A receptor antagonist L-364,4718 (1 to 100nM on 8 neurones) produced a rapid antagonism of both the inhibitory and excitatory CCK responses. On neurones which were excited by CCK, using the equation "Dose Ratio-1=[antagonist]/equilibrium constant" the equilibrium constant for L-364.718 was calculated to be 0.127±0.043nM (n=4). Bath application of the specific CCK_B antagonist L-365,260 on 4 neurones (10uM for 15 to 20 minutes) had a weak antagonist action on the CCK response. Bath application of prazosin (1uM) or spiperone (10uM) which abolished the phenylephrine and 5-HT responses had no effect on the response to CCK.

The equilibrium constant obtained with L-364,718 was similar to that obtained in binding studies to CCK_A binding sites in the pancreas (Chang and Lotti 1986). This suggests that CCK excites a subpopulation of neurones in the dorsal raphe by a CCK_A receptor. This is supported by the lack of action of the CCK_B agonist pentagastrin and weak action of the potent CCK_B receptor antagonist L-365,260 (Lotti and Chang 1989). The persistence of the CCK response in the presence of prazosin and spiperone, shows that the mechanism of action of CCK was unlikely to involve either noradrenaline or 5-HT.

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350P CHOLECYSTOKININ MODULATES THE RELEASE OF DOPAMINE FROM THE ANTERIOR AND POSTERIOR NUCLEUS ACCUMBENS BY TWO DIFFERENT MECHANISMS

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It has been shown by dicrete lesioning studies that the coexistence of cholecystokinin (CCK) and dopamine in A10 neurones is confined to those neurones which project into the posterior portion of the nucleus accumbens (Studler et al.,1981). CCK has been found to have different effects on dopamine mediated behaviours (Crawley et al.,1985), dopamine stimulated adenylate cyclase (Studler et al., 1986) and on dopamine release (Voigt et al.,1986) in the anterior compared to the posterior nucleus accumbens. In this study we have used receptor specific antagonists to characterise the receptors involved in the CCK modulation of endogenous dopamine release from slices of anterior and posterior rat nucleus accumbens. CCK sulphated octapeptide (CCK8S) (1-10uM), but not CCK unsulphated octapeptide (CCK8US) or pentagastrin caused a dose-dependent increase in 35mM potassium stimulated endogenous dopamine release from slices of posterior nucleus accumbens. This effect was blocked by the CCK_A receptor antagonist L364,718 (10nM) but not by the CCK_B receptor antagonist L365,260 (0.1uM). In slices of anterior nucleus accumbens however CCK8S and CCK8US (0.1-1.0uM) caused an inhibition of potassium stimulated dopamine release. This effect was blocked by L365,260 (0.1uM), but not by L364,718 (10nM) suggesting that it was mediated by a CCK_B receptor. These data suggest that CCK causes a site specific increase or decrease in dopamine release from nucleus accumbens by acting on either CCK_A or CCK_B receptors.

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351P ELECTROCONVULSIVE SHOCK INCREASES DOPAMINE D_1 RECEPTOR-MEDIATED ADENYLATE CYCLASE ACTIVITY IN RAT STRIATAL HOMOGENATES

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Identification of dopamine (DA) D1 and D2 receptors prompted a reevaluation of the effects of electroconvulsive shock (ECS) on this system. Martin et al. (1988) found D1 receptors unaltered after ECS, but Newman & Lerer (1989) claimed that ECS increased D1 function. We now report the effects of ECS on D1-linked adenylate cyclase in rat striatum (CPU).

Halothane anaesthetised male CD rats (100-125 g) received either one ECS (200 V, 2 s) or 5 ECS over 10 days. CPU were removed 24 h later and the adenylate cyclase response to DA (1-100 μM) in membranes was determined (Kebabian et al, 1972), with and without 5 μM sulpiride (SULP). The apparent EC50 and Vmax were determined by non-linear regression analysis.

Neither a single nor repeated ECS affected the adenylate cyclase response to DA which activates stimulatory D1 and inhibitory D2 receptors (Table 1). In the presence of sulpiride, which blocks D2 receptors, the EC50 for DA was decreased and the Vmax was increased after one ECS. After repeated ECS, only the Vmax was elevated (Table 1). Basal adenylate cyclase activity was unaltered after acute or repeated ECS.

Table 1: The effects of single or repeated ECS on DA-stimulated adenylate cyclase activity in rat CPU.

	ECS x1				ECSx5			
Stimulation	EC50 (μM)		Vmax		EC50 (μM)		Vmax	
	Control	Test	Control	Test	Control	Test	Control	Test
DA	5.6±1.4	5.2±1.3	44.8±3.8	39.7±3.7	7.8±1.1	11.3±2.4	39.8±3.6	45.6±6.9
DA+SULP	4.4±0.7	2.4±0.3*	52.7±3.8	72.0±3.4***	6.1±0.8	6.8±1.0	33.3±2.0	46.8±4.4*

Mean±s.e.mean (n=6-9 individual rats). Vmax pmol/2.5mg wet wt./2.5min. *P<0.05, ***P<0.005 vs. control.

We agree with Newman & Lerer (1989) that the overall response to DA is unaltered by single or repeated ECS and that specific D1-stimulated adenylate cyclase is increased by the latter. Our finding of increased D1 function after one ECS was not reported by Newman & Lerer (1989). However, their results show an increase which was not statistically significant. Thus, although ECS does not increase D1 receptor binding (Martin et al; 1988), it clearly increases the functional response to specific stimulation of this receptor.

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352P THE ACTIVITY OF THE DOPAMINE AGONIST N-0437 AND EIGHT ESTER PRODRUGS AFTER ORAL AND TRANSDERMAL ADMINISTRATION IN THE 6-OHDA TURNING MODEL

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The potent and selective D2-agonist N-0437 (2-(N-propyl-N-2-thienylethylamino)-5-hydroxytetralin) undergoes considerable first-pass metabolism due to glucuronidation of the phenol group after oral administration. In an attempt to improve the bioavailability, nine ester prodrugs of N-0437 were synthesized, ie. the methyl-, ethyl-, isobutyryl-, pivaloyl-, phenyl-, 2,4-di-methylphenyl, 2-aminophenyl- and 2-methoxyphenyl-analogues. To examine the hydrolysis rates of these compounds in vitro studies were performed in rat blood. The results and the analytical method for the determination of the aminotetralins is described elsewhere (1, 2). The prodrugs showed a very diverse pattern of hydrolysis rates. The in vivo activities were determined by testing N-0437 and the prodrugs after oral and transdermal administration in rats with unilateral 6-OHDA lesions of the striatum. The resulting contralateral turning was used to measure the activity of the compounds. By calculating the area under the curve (AUC), of the time-effect curves of N-0437 and the prodrugs in several time intervals an improved duration of action was found for those prodrugs which have a slow in vitro hydrolysis rate after oral administration.

		AUC			
Treatment	Route	1-5 h	6-10 h	11-15 h	1-20 h
vehicle	p.o.	50±25	56±20	67±18	221±59
N-0437	p.o.	1610±220 **	379±101	133±42	2195±309 **
	t.d.	371±29	815±67 **	967±45 **	2982±94 **
isobutyryl	p.o.	2356±324 **	1166±357 **	684±209 **	4764±938 **
	t.d.	1084±182 **	1092±194 **	797±139 **	3576±600 **
2-aminophenyl	p.o.	1182±405 **	1010±209 **	335±59 **	2810±574 **
	t.d.	53±28	25±10	36±9	129±25

Effect of oral (p.o.) and transdermal (t.d.) application (50 μmol/kg)
(mean±SEM; N-10; *p<0.05, **p<0.01 vs. vehicle, Newman-Keuls test)

However no activity of these compounds could be found after transdermal application. The isobutyryl compound showed an increase of activity in all time intervals after oral administration. The methyl-, the ethyl- and the isobutyryl analogues which are prodrugs with a relatively fast hydrolysis rate were found to have interesting profiles following transdermal application.

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353P ATYPICAL NEUROLEPTIC-LIKE PROPERTIES OF NEUROTENSIN: AN *IN VIVO* NEUROCHEMICAL STUDY

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Many central effects produced by neurotensin (NT) resemble those of neuroleptics. However, contrary to typical neuroleptics, NT does not inhibit stereotypy induced by dopamine stimulating drugs (Ervin et al., 1981) and it has been suggested that the neuropharmacological effects of NT resemble more those of atypical neuroleptics (Jolicoeur et al., 1985). One way of discriminating between typical and atypical neuroleptics is to measure the level of DOPAC in the striatum after treatment with the neuroleptics and the non-amphetamine stimulant amfonelic acid. It has been reported that amfonelic acid potentiates the *ex vivo* increase of striatal DOPAC level induced by typical but not by atypical neuroleptics (Waldmeir et al.,1985). In the present study, we have examined whether amfonelic acid can be used to discriminate between typical and atypical neuroleptics and NT *in vivo* by monitoring extracellular DOPAC in the striatum using differential pulse voltammetry with carbon fibre micro-electrodes.

Male Wistar rats (250-280 g) were anaesthetised with chloral hydrate (400 mg/kg i.p.) and stereotaxically implanted with one electrically-pretreated 12 μm carbon fibre electrode in the striatum. Extracellular DOPAC was recorded every five minutes during a stabilisation period of 45 min before drug administration. Saline (1ml/kg s.c.) or amfonelic acid (2.5 mg/kg s.c.) were administered and 5 min later animals received haloperidol (1 mg/kg s.c.), perphenazine (10 mg/kg s.c.), clozapine (30 mg/kg i.p.), thioridazine (20 mg/kg s.c.) or NT (10 μg i.c.v.). Extracellular DOPAC was recorded for 90 min after drug administration and the results are expressed as percentage differences compared with baseline value. Data were assessed by two way analysis of variance for repeated measures.

Amfonelic acid alone (n=6) did not alter extracellular DOPAC level in the striatum. Both haloperidol and perphenazine (n=6) increased extracellular striatal DOPAC by about 85% 90 min after administration but when amfonelic acid was given 5 min before the neuroleptics, the increase in DOPAC was greatly potentiated (227% and 153% respectively 90 min after injection). However, while clozapine, thioridazine and NT (n=6) also significantly increased the extracellular level of DOPAC in the striatum (51%, 25% and 27% respectively after 90 min), the administration of amfonelic acid 5 min before totally prevented the rise in DOPAC induced by the atypical neuroleptics and NT.

In conclusion, the *in vivo* data confirm that amfonelic acid is a valuable tool to neurochemically differentiate potential typical from atypical neuroleptics. Using this model, it appears that the atypical neuroleptics and NT produced similar *in vivo* neurochemical effects. It remains to be determined why amfonelic acid antagonised the rise in DOPAC produced by clozapine, thioridazine and NT.

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354P ACUTE CGS 8216 REVERSES, AND CONCURRENT VERAPAMIL PREVENTS, DIAZEPAM WITHDRAWAL-INDUCED ANXIETY IN RATS

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Given acutely, FG 7142, a benzodiazepine (BDZ) receptor partial inverse agonist, reversed the anxiogenic and proconvulsant effects of diazepam (DZP) withdrawal in rats (Hitchcott et al, 1989). However, another partial inverse agonist, CGS 8216 (CGS), has been reported to enhance certain physical signs of DZP withdrawal (Zharkovsky, 1990). Here we have used the elevated plus-maze to examine the effect of acute CGS administration on the anxiogenic DZP withdrawal response (File, 1989) and also the effect of verapamil (VER), a calcium channel antagonist, given concurrently with chronic DZP treatment, on this withdrawal response.

Prior to the start of chronic drug treatment male hooded Lister (200-250g) rats were implanted with bilateral cannulae into the lateral ventricles. The rats then received daily i.p. injections of DZP (4mg/kg) or vehicle (water/Tween) for 28 days and a second daily injection of either VER or vehicle given i.p. 5/7 days and i.c.v. 2/7 days (10mg/kg i.p.; 10μg/rat i.c.v.). Rats were tested on the elevated plus-maze 42h after the last DZP injection. VER (10mg/kg i.p.) was administered 24h after the last DZP injection and again 30mins before testing. CGS (5mg/kg i.p.) was administered once, 30mins before testing.

DZP withdrawal significantly reduced the percentage (%) time spent on open arms of the maze compared with controls indicating an anxiogenic response (Con: 18.0±2.2, With: 11.0±2.8 $p<0.05$). Concurrent VER prevented this withdrawal response (With+VER: 36.6±3.6 $p<0.01$ vs Con) but was without effect in vehicle-treated animals (VER alone: 15.0±5.0). In vehicle-treated rats CGS significantly decreased the % time spent on open arms of the maze compared with controls (CGS alone: 2.3±1.6 $p<0.01$) but failed to enhance the anxiogenic response in DZP-withdrawn rats. Instead, a significant withdrawal X CGS interaction ($p<0.01$) indicated a reversal of this withdrawal response (With+CGS: 16.5±5.6).

The contrasting effects of CGS on the physical signs (Zharkovsky, 1990) and the anxiogenic effect of DZP-withdrawal (present study) suggest that separate neurochemical mechanisms may underlie different BDZ withdrawal responses. Concurrent calcium channel antagonist administration during chronic ethanol treatment has previously been shown to prevent withdrawal-induced convulsive behaviour (Whittington & Little, 1988). Our results suggest that calcium channel function may also be altered by chronic BDZ treatment and/or its withdrawal.

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355P SPECIES DIFFERENCES IN THE SENSITIVITY TO [³H]-PK11195, A POTENTIAL MARKER OF NEURONAL DAMAGE

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The benzodiazepine receptor binding sites characterised by high affinity for the ligands [^{3}H] PK 11195 and [^{3}H] RO 5 4864 differ from the classical central benzodiazepine receptor in terms of pharmacological specificity, subcellular and anatomical distribution, although these sites are also located within the CNS (Anholt et al, 1985). In this study we have examined the properties of these sites in gerbil and rat brain using the selective ligand [^{3}H] PK 11195.

Tris washed membranes from rat and gerbil cortex both display high affinity to [^{3}H] PK 11195 (Kds 0.21±0.05, and 0.17±0.02 nM respectively) but a greater number of sites are labelled in the gerbil cortex (1360±71 fmol/mg protein) than in the rat (254±21fmol/mg). Hippocampal membranes from rat and gerbil are labelled with similar affinity but more sites are labelled in the gerbil (1430±111 fmol/mg) than the rat (196±31fmol/mg). Competition studies in cortical membranes show that RO 5 4864 displaces [^{3}H] PK 11195 with high affinity (pK_i 8.48±0.02) in rat but with lower affinity in the gerbil (pK_i 6.70±0.12), a property shown to a lesser extent with flunitrazepam.

Table 1. Affinity values for compounds at the [^{3}H] PK 11195 binding site in cortical membranes.

Compound	Rat cortex		Gerbil cortex		
	pK_i	nH	pK_i	nH	
PK 11195	9.28 ±0.08	1.02	9.38 ±0.15	1.13	Values represent mean,
RO 5 4864	8.48 ±0.02	0.92	6.70 ±0.12	0.91	± s.e.mean (n=4)
Dipyridamole	7.38 ±0.12	1.08	7.55 ±0.10	1.03	
Diazepam	7.25 ±0.15	0.90	5.54 ±0.11	0.90	
Flunitrazepam	7.14 ±0.09	0.93	5.42 ±0.03	0.87	

A brief period of forebrain ischaemia in the gerbil was induced by bilateral occlusion of the common carotid arteries as previously described (Alps et al, 1988) followed by 7 days recovery after which time brain areas were assayed, or sections cut for autoradiography studies. In both experiments an increase in specific [^{3}H] PK 11195 was produced as a result of the ischaemic period followed by recovery. This effect was most evident in membranes derived from the hippocampus, and in the hippocampal area on autoradiographic sections. In control hippocampal membranes the Bmax for [^{3}H] PK 11195 was 1.43±0.11 pmol/mg and this increased to 2.16±0.17 pmol/mg following ischaemia with recovery.These results show that [^{3}H] PK 11195 may be a useful marker for ischaemic damage, but the marked species differences observed in its profile require further investigation.

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356P EVALUATION OF TRICYCLIC ANTIDEPRESSANTS IN THE ELEVATED PLUS-MAZE IN RATS: ANXIOLYTIC EFFECT OF DOTHIEPIN AND DOXEPIN

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The elevated plus-maze is a rodent model of anxiety capable of detecting the anxiolytic activity of the benzodiazepines (Pellow et al, 1985). We have examined a range of tricyclic antidepressants to assess whether this test is selective for anxiolytic drug action.

Male Sprague Dawley rats (170-200g; Charles River) were orally administered drugs or vehicle 1 h prior to placing in the centre of a wooden elevated plus-maze. Open and closed arm dimensions were respectively 45 x 10 cm and 45 x 10 x 40 cm. Control, vehicle-treated rats rarely entered the open arms but shuttled freely between the closed arms (Table 1). Oral administration of chlordiazepoxide and diazepam each caused a dose-dependent increase in the open arm entries and in the time spent on the open arm (Table 1). Both open arm measures were similarly increased by the tricyclic antidepressants dothiepin and doxepin. In contrast, amitriptyline had only a weak effect on open arm entries, and imipramine and desipramine had no effect on either open arm measure (Table 1). Total arm entries were reduced only by desipramine (10 mg/kg, -25%).

Table 1 Effect of benzodiazepines and tricyclic antidepressants in the elevated plus-maze in rats

Treatment	Oral doses tested (mg/kg)	Dose (mg/kg)	Open arm entries (mean ± SEM)	Closed: open ratio	Time on open arms (mean ± SEM, sec)
Vehicle	-	-	0.8 ± 0.1***	19	6 ± 1***
Chlordiazepoxide	0.3 - 10	10	9.2 ± 0.7***	2	165 ± 21***
Diazepam	0.1 - 10	10	6.2 ± 0.5***	2	117 ± 25**
Dothiepin	0.1 - 30	10	6.8 ± 0.5***	3	54 ± 6**
Doxepin	0.1 - 30	10	5.2 ± 0.3*	3	51 ± 7
Amitriptyline	1 - 30	30	2.2 ± 0.4	7	19 ± 5
Imipramine	1 - 30	30	1.0 ± 0.2	16	9 ± 1
Desipramine	0.3 - 10	10	1.3 ± 0.2	10	9 ± 2

Vehicle controls have been combined for clarity; statistical significance (ANOVA; ***p<0.001, **p<0.01, *p<0.05) assessed against appropriate control only. n=6 rats/dose.

The anxiolytic effect of dothiepin and doxepin in the elevated plus-maze does not necessarily question the specificity of the model for detecting anxiolytics. Indeed, the anxiolytic activity of dothiepin and doxepin, but not imipramine and desipramine, appears to support the clinical indication of the former drugs being of benefit in patients suffering from depression with anxiety (Tyrer, 1989).

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357P EFFECT OF ISOLATION-REARING ON PERFORMANCE IN THE ELEVATED PLUS-MAZE AND OPEN FIELD BEHAVIOUR

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Isolated rats demonstrate many behavioural disturbances such as increased locomotor activity, neophobia, and deficits in both learning and spatial memory tasks (Morgan *et al.*, 1975). Neophobia, and some of the other behavioural differences observed in isolation-reared animals, are thought to be related to increased anxiogenic behaviour and a raised level of anxiety is observed when the isolation-reared rats are placed in a novel enviroment (Morinan and Parker, 1985). The aim of this study was to investigate the effects of social isolation on rats using the elevated plus maze test of anxiety (Handley and Mithani, 1984) and open field behaviour.

Male, Lister hooded rats (Harlan Olac Ltd.) were caged either individually or in social groups of five at weaning (21 days of age) and for the duration of the experiment. 30 days post-weaning the behavioural tests were undertaken using the elevated plus-maze which consisted of two open and two enclosed arms which were raised 70cm above the ground. The percentage time and percentage entries into the open arms, actual time on the end of the open arms and number of rears in the open and closed arms were used as measures of anxiety. The total number of arm entries was used as an index of locomotor activity. Alternate isolation- and socially-reared animals were placed in the centre of the maze facing an enclosed arm and monitored for a 5 min period. One week later the same animals were exposed to the open field test which consisted of an open-topped cylindrical drum divided up into concentric circles crossed by radial lines producing areas of equal size. Isolation- and socially-reared rats were placed alternately in the central circle of the open field and recorded on video for a 5 min period. The video was analysed for number of line crossings, time spent in the outer 2 circles and number of rears once all of the data had been collected.

Isolation-reared rats spent significantly less time on the open and the end of the open arm (Isol: 48.1±7.1, Soc: 75.5±9.8 and Isol: 24.7±4.5, Soc: 36.6±5.1, $p<0.05$, $n=20$ respectively) and had reduced percentage open/total time (Isol: 21.3±3.3, Soc: 31.3±3.9, $p<0.05$, $n=20$) but no difference in the percentage open/total entries, number of rears or total arm entries. Isolation-reared rats spent significantly more time in the outer 2 circles of the open field (Isol: 254.9±4.9, Soc: 227.2±5.2, $p<0.01$, $n=20$) than the socially-reared animals. There was no difference in the number of rears and the total number of line crossings between the two groups.

The behavioural data suggest that rats reared in isolation immediately post-weaning exhibit a more anxiogenic profile than socially-reared animals when placed in a novel enviroment; this effect is not a result of alterations in locomotor activity observed in these animals.

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358P EVIDENCE FOR SUBTYPES OF PERIPHERAL PREJUNCTIONAL α_2-ADRENOCEPTORS

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Alpha-2 adrenoceptors have been subdivided into alpha-2A, -2B and -2C based on ligand binding studies (Bylund, 1988), although functional correlates of these sites have proved difficult to establish. We have investigated the effects of a series of 10 antagonists at prejunctional alpha-2 adrenoceptors in rat vas deferens and atria and correlated potencies with affinities for the alpha-2A binding site of human platelet and alpha-2B binding site of rat kidney.

In prostatic portions of rat vas deferens, antagonist potency was assessed as the ability to antagonise the prejunctional inhibition by the alpha-2 adrenoceptor agonist xylazine of the isometric twitch to a single electrical pulse. In rat isolated atria and in prostatic portions of rat vas deferens, antagonist potency was assessed as the ability to produce 30% potentiation of stimulation-evoked overflow of tritium (EC_{30}) in tissues pre-incubated with [^{3}H]-noradrenaline. In human platelet membranes and in rat kidney cortex membranes, K_i values were obtained for antagonists from their ability to displace [^{3}H)-yohimbine binding.

The correlation of the alpha-2A binding site of human platelet was better with antagonist pA_2 in rat vas deferens (slope = 1.02±0.13, r = 0.94, $p<0.001$) than with antagonist EC_{30} in rat atria (slope = 0.68±0.17, r = 0.81, $P<0.01$). The correlation of the alpha-2B binding site of rat kidney was better with antagonist EC_{30} in rat atria (slope = 0.98±0.11, r = 0.95, $P<0.001$) than with antagonist pA_2 in rat vas deferens (slope = 1.15±0.22, r = 0.88, $P<0.001$). Three antagonists (prazosin, ARC 239 and chlorpromazine) showed selectivity for the prejunctional receptors of atria, with EC_{30} values of 6.97±0.09, 7.40±0.32 and 6.53±0.12, respectively (-log M), as compared with K_B values in rat vas deferens of 5.88±0.09, 5.71±0.10 and 5.69±0.07. These antagonists also showed low potency in rat vas deferens when potency was assessed as an EC_{30} with values of $\leq$ 6.0, 6.17±0.25, and <6.0, respectively.

In conclusion, the prejunctional alpha-2 adrenoceptors of rat vas deferens and rat atria differ, and may be tentatively identified as alpha-2A and alpha-2B, respectively.

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359P NEURONALLY-RELEASED NORADRENALINE ACTS AT DIFFERENT α_1-ADRENOCEPTORS TO EXOGENOUS NORADRENALINE IN RAT VAS DEFERENS

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The time course of the in-vitro mechanical response of the whole rat vas deferens to twin pulse electrical field stimulation (TPFS - 0.8ms width, 40V, 3s inter-pulse interval, via parallel platinum electrodes) was investigated using a computerised data collection system (Marshall & Spriggs, 1985). At a 3 second stimulus interval the mechanical responses to each impulse are discrete.

Chloroethylclonidine (CEC), an α_{1B}-adrenoceptor antagonist (Han et al.,1987), progressively inhibited the second phase of responses to the 1st stimulus of TPFS during a 30 min exposure. The effect was concentration-related (10^{-7} to $3x10^{-6}$M), with the highest concentration achieving an almost complete block of the second phase. In contrast CEC (10^{-6}M) failed to modify the dose response curve to exogenously applied NA ($3x10^{-8}$ to $3x10^{-4}$M). Nifedipine (10^{-5}M) inhibits the first phase of the response to each stimulus but leaves the second (noradrenergic) phase of the response to the first stimulus unaffected and reveals a small residual second phase component in the response to the second stimulus. However, nifedipine (10^{-5}M) completely abolished the responses to exogenous NA (10^{-8} to $5x10^{-4}$M). In the presence of nifedipine (10^{-5}M) CEC (10^{-7} to $3x10^{-6}$M) produced concentration dependent reductions in the residual (noradrenergic) components of the responses to both stimuli.

Our results lead us to suggest that the adrenoceptors activated by neuronally-released NA (following TPFS) are different to those activated by exogenous noradrenaline. Those adrenoceptors responsible for the noradrenergic component of the TPFS response appear to be of the α_{1B} sub-type with the excitation contraction coupling probably mediated by an IP_3 mechanism (Han et al (1987) while the response to exogenous NA is produced by activation of adrenoceptors of the α_{1A} sub-type which are coupled through dihydropyridine-sensitive calcium channels. It is possible that the α_{1B} adrenoceptors may be restricted to the synaptic regions while the α_{1A} adrenoceptors are widely spread over the whole smooth muscle membrane and are thus more accessible to exogenous agonists .

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360P FUNCTIONAL COMPARISONS OF L-365,260 AND OTHER GASTRIN/CCK RECEPTOR ANTAGONISTS IN GASTRIC MUCOSA AND ILEUM *IN VITRO*

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We previously reported that the rank orders of agonist potency for gastrin/cholecystokinin (CCK) peptides to stimulate acid secretion in rat isolated gastric mucosa (RGM) and contract guinea-pig isolated ileum longitudinal muscle-myenteric plexus (GPI-LMMP) are consistent with the responses being mediated by gastrin and CCK_A receptors respectively (Spraggs and Patel, 1989). These responses have now been characterised further using the antagonists, L-365,260, L-364,718, lorglumide and Boc-ßAWLD-O$(CH_2)_2$Ph. RGM and GPI-LMMP were prepared and used as described previously (Spraggs and Patel, 1989). The effects of the antagonists on responses to pentagastrin (PTG) in RGM and CCK-8 in GPI-LMMP were determined by constructing concentration response curves for the agonists before and after 30 min equilibration of antagonist in individual tissues. All four antagonists produced concentration related rightward displacements of curves in both tissues and pA_2 values and Schild slopes (mean ± SEM), determined by linear regression of 3 or 4 concentrations for each antagonist (n=12 to 16) are shown below.

Antagonist	RGM		GPI-LMMP	
	pA_2	slope	pA_2	slope
Boc-ßAWLD-O$(CH_2)_2$Ph	6.53±0.24	1.85±0.41*	6.12±0.30	1.23±0.31
lorglumide	5.47±0.21	1.39±0.28*	7.50±0.13	1.50±0.13*
L-364,718	5.77±0.30†	-	10.40±0.30	0.94±0.13
L-365,260	7.62±0.10	1.11±0.11	7.72±0.33	1.14±0.24

*: Slope value significantly greater than unity ($p<0.05$). †: pK_B estimate at 10µM (n=4)

Boc-ßAWLD-O$(CH_2)_2$Ph was a non-selective antagonist in both RGM and GPI-LMMP. In contrast, lorglumide and L-364,718 were potent antagonists of CCK-8 responses in GPI-LMMP, with selectivity ratios of 100 and 10000 fold compared with their affinity in RGM and consistent with their reported CCK_A receptor selectivity (Freidinger, 1989). L-365,260 was the most potent antagonist of PTG in RGM, with a pA_2 value consistent with affinity estimates in other functional gastrin/CCK_B receptor mediated systems (Huang et al, 1989; Kemp et al, 1989). However, L365,260 was approximately 50 times less potent in RGM than reported in gastrin/CCK_B receptor binding assays (Freidinger, 1989) and was a non-selective antagonist in RGM and GPI-LMMP. These studies are consistent with the view that responses in RGM and GPI-LMMP are mediated by gastrin and CCK_A receptors respectively. They also suggest that in functional studies, L-365,260 used alone may not be a useful tool to differentiate gastrin from CCK_A receptors.

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361P EFFECTS OF ERYTHROCYTE EXTRACTS FROM GENETICALLY HYPERTENSIVE RATS ON VASCULAR SMOOTH MUSCLE AND ON CALCIUM UPTAKE BY HUMAN RED CELLS

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Partially purified erythrocyte extracts from spontaneously hypertensive rats produce hypertension when administered to normotensive rats and increase Ca uptake by rat aortic rings (McCumbee & Wright, 1985; Wright et al., 1986). In this study extracts were purified and their effect on Ca uptake in human red blood cells (HRBC) and on vascular smooth muscle contractility was investigated.

Erythrocytes were obtained from 16 week old New Zealand Genetically Hypertensive (NZGH) rats. Matched normotensive rats were used as controls. The cells were dialysed as described by McCumbee & Wright (1985) and the lyophilised dialysate was separated into 4 fractions using Sep. Pak cartridges preactivated with acetonitrile. The effect of the fractions on Ca uptake was tested on HRBC obtained from normal human volunteers. Washed cells were suspended in buffer containing 45Ca with or without the addition of fractions and incubated at 37OC. At various time intervals samples of cell suspensions were taken and 45Ca activity was determined. Fraction 0 of NZGH rats increased calcium uptake by HRBC by a factor of 24.6. There was only a two-fold increase of Ca uptake by HRBC exposed to fraction 0 of normal rats while fractions 1, 2 and 3 from normo- and hypertensive rats had no effect.

Fractions from hyper- and normotensive rats had a similar effect on smooth muscle. Fraction 0 increased the contractility of rat portal vein preparations by 96%, fraction 1 by 124%, fraction 2 by 39% while fraction 3 had no effect. Only fraction 1 induced contractions of aortic rings, with the response ranging 0.34 to 0.46 g tension. Since the pattern of smooth muscle response to fraction 1 was similar to responses evoked by spasmogens of low molecular mass, the High Performance Liquid Chromatography of fraction 1 was compared with that of adrenaline, noradrenaline, 5-hydroxytryptamine, angiotensin II and tyramine. The chromatograph of fraction 1 was different from those of the standard spasmogens.

Only fraction 0 of hypertensive rats had a marked effect on Ca uptake by HRBC but was without effect on rat aorta. The effect of fraction 1 on vascular smooth muscle could indicate an involvement in the development of hypertension. The fact that it is also present, but apparently inactive, in normotensive rats could be explained by a modifying action of fraction 0 whose pronounced effect on Ca uptake by HRBC is present only in erythrocyte extracts from hypertensive rats.

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362P MUSCARINIC RECEPTOR-MEDIATED PHOSPHOINOSITIDE HYDROLYSIS IS INHIBITED BY ISOPRENALINE AND IBMX IN BOVINE TRACHEAL SMOOTH MUSCLE

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Stimulation of β_2-adrenoceptors in airway smooth muscle (ASM) has previously been shown to inhibit phosphoinositide (PI) hydrolysis induced by histamine (Hist) and 5-hydroxytryptamine (5HT) but not carbachol (CCh) or methacholine (Madison and Brown, 1988; Hall and Hill, 1988). This has led to the proposal that these responses are mediated by different post-receptor signal transduction mechanisms. However, since there exists a substantial muscarinic receptor reserve in bovine tracheal smooth muscle (BTSM) for [^{3}H]inositol phosphate ([^{3}H]-InsP) production (Meurs et al. 1989) we have re-examined the effect of isoprenaline (Iso) and, the non-selective phosphodiesterase inhibitor, 3-isobutyl-1-methyl-xanthine (IBMX) on PI hydrolysis using full and partial muscarinic agonists. Aliquots of BTSM slices (50µl, 300 x 300 µm) were labelled with 1.5 µCi myo[^{3}H]inositol in 2ml multiwell plates containing 1ml supplemented MEM (37°C, 5% CO_2 incubator) for 24 hrs and stimulated for 30 min in the presence of 10mM LiCl. Total [^{3}H]InsP accumulation was determined in neutralised-TCA extracts by anion exchange chromatography. Iso (10µM) and IBMX (1mM) were added 20 min prior to agonists. Under these conditions no agonist-stimulated incorporation of myo-[^{3}H]inositol into the inositol lipids was observed. CCh, oxotremorine (Oxo), pilocarpine (Pilo) and Hist stimulated [^{3}H]InsP accumulation in a concentration-dependent manner (CCh 100%, EC_{50} 3.5 µM; Oxo 63 ± 8%, EC_{50} 0.56 µM; Pilo 34 ± 9%, EC_{50} 3.1 µM; Hist 49 ± 8%, EC_{50} 7.6 µM; mean ± SEM, 3-7 experiments). Pre-treatment of BTSM slices with ISO attenuated Pilo and Hist-stimulated [^{3}H]InsP accumulation (43 ± 3%, 47 ± 6% inhibition respectively at 1 mM [agonist] both $p<0.05$) without influencing the EC_{50} values and had no effect on CCh or Oxo responses. In contrast, IBMX significantly inhibited Oxo, Pilo and Hist-stimulated accumulation at all agonist concentrations (35 ± 9%, 71 ± 5%, 84 ± 8% inhibition respectively at 0.1 mM [agonist]) while attenuating CCh-stimulated [^{3}H]InsP accumulation only at low CCh concentrations (CCh 1µM 91 ± 7%, 10µM 58 ± 13% inhibition). In addition, CCh and Hist-stimulated [^{3}H]InsP accumulation were non-additive (CCh 1mM 100%, Hist 1mM 44 ± 3%, CCh plus Hist 109 ± 6%, $p<0.05$) and the Oxo and Hist responses were inhibited to a similar degree by chelation of extracellular Ca^{2+} using 2mM EGTA ($[Ca^{2+}]$ ~6µM; inhibition Oxo 20.9 ± 3.9%, Hist 32.5 ± 5.2% $p>0.05$).
These data demonstrate that [^{3}H]InsP accumulation in response to the partial muscarinic agonist Pilo is significantly inhibited by Iso and that IBMX additionally affects both Oxo and low concentration CCh-stimulated PI hydrolysis. Together with the non-additivity of the Hist and CCh responses and the similar sensitivity of Hist and Oxo effects to extracellular $[Ca^{2+}]$ this may indicate shared signal-transduction pathways. Furthermore, this suggests that muscarinic receptor stimulation of PI metabolism in BTSM is complex and that inhibition by ß-adrenoceptor activation can only be revealed when a weak partial muscarinic agonist is used as a stimulus.

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363P IS THE TRANSIENT ACCUMULATION OF INOSITOL(1,4,5)TRISPHOSPHATE IN MUSCARINIC-STIMULATED AIRWAY SMOOTH MUSCLE DUE TO TRANSIENT FORMATION OR ENHANCED METABOLISM?

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It is well established that the stimulation of Ca2+ mobilizing receptors in a range of tissues, including bovine tracheal smooth muscle (BTSM), results in a transient accumulation of the second messenger inositol(1,4,5)trisphosphate (Ins(1,4,5)P3). This may result from either transient hydrolysis of phosphatidylinositol(4,5)bisphosphate (PtdIns(4,5)P2) or stimulation of the metabolism of Ins(1,4,5)P3 via the 3-kinase or 5-phosphatase enzymes. We now demonstrate that in BTSM carbachol stimulation results in sustained PtdIns(4,5)P2 hydrolysis, and hence Ins(1,4,5)P3 formation, at a time when Ins(1,4,5)P3 concentr--ation has returned to basal values indicating receptor-stimulated Ins(1,4,5)P3 metabolism.
BTSM slices (300 x 300um) were labelled with 0.5 uM myo-[3H]inositol in oxygenated Krebs-Henseleit buffer at 37°C for 60min and aliquots stimulated with 0.1mM carbachol. [3H]InsP1-4 and Ins(1,4,5)P3 mass were determined in neutralised-TCA extracts by anion exchange chromatography and radioreceptor assay (Challiss et al, 1988) respectively. The phosphoinositides were extracted from the tissue pellet using acidified chloroform-method and, following deacylation, [3H]glycerophosphorylinositol ([3H]GroPtdIns), [3H]GroPtdInsP and [3H]GroPtdInsP2 were separated using Dowex AG1-X8 columns. PtdIns(4,5)P2 mass was determined by boiling an aliquot of the same lipid extract in 1M KOH (30min) which generates Ins(1,4,5)P3, Ins(2,4,5)P3 and Ins(4,5)P2 in an experimentally determined fixed molar ratio of 66:20:14, and assaying the desalted, butan-1-ol/petroleum ether washed product for Ins(1,4,5)P3 mass.
The kinetic data from these experiments is detailed in Table 1. Preliminary experiments examining the deacylated ([3H]GroPtdIns(P)) and deglycerated ([3H]InsPx) products of control and carbachol-stimulated (0.1mM, 10min) [3H]inositol labelled BTSM slices and their sensitivity to human erythrocyte 5-phosphatase treatment, using hplc, demonstrated that [3H]PtdInsP2 was exclusively [3H]PtdIns(4,5)P2. The stimulated incorporation of [3H]inositol into PtdInsP2 over the initial 10min period indicates that the sustained decline in PtdIns(4,5)P2 mass, which was fully and rapidly reversed by 10uM atropine (t1/2 2.4min), was not due to agonist stimulated conversion of PtdInsP2 to PtdInsP.

Table 1	Time (s)	0c*	5	60	60c*	300	600	600c*	1800	1800c*
Ins(1,4,5)P3	(pmol/mg protein)	11.6	22.3	8.0	10.5	7.2	-	-	9.4	10.8
[3H]InsP3	(dpm)*1	227	264	398	268	1067	2015	268	2965	193
[3H]PtdInsP2	(dpm)	1620	1353	815	-	3452	5197	2342	6880	4413
PtdIns(4,5)P2	(nmol)	1095	732	427	1216	401	478	1137	526	1110

Means of 3 experiments. dpm & PtdIns(4,5)P2 values/50ul BTSM slices; *control; *1>80% Ins(1,3,4)P3 by 60s.
These data show that carbachol stimulation of BTSM causes transient Ins(1,4,5)P3 accumulation despite sustained PtdIns(4,5)P2 hydrolysis indicating enhanced metabolism of Ins(1,4,5)P3 under these conditions.

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364P ZAPRINAST DOES NOT INFLUENCE METHACHOLINE-INDUCED TONE OR INOSITOL(1,4,5)TRISPHOSPHATE ACCUMULATION IN BOVINE AIRWAY SMOOTH MUSCLE

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The proposal that phosphatidylinositol(4,5)bisphosphate hydrolysis and inositol(1,4,5)trisphosphate (Ins-(1,4,5)P_3) formation is involved in agonist-induced airway smooth muscle (ASM) contraction has been challenged by the recent demonstration in guinea pig isolated tracheal rings, that zaprinast, a cGMP-selective phosphodiesterase inhibitor, abolishes both histamine and methacholine(MCh)-stimulated Ins(1,4,5)P_3 accumulation, without influencing the generation of tone by either agonist (Langlands et al. 1989). In view of the fundamental importance of this observation, we have re-evaluated the effect of zaprinast on MCh-induced tone and Ins(1,4,5)P_3 and cGMP accumulation in a near homogeneous ASM preparation.
Bovine tracheal smooth muscle (BTSM) slices (300x300μm) were pre-incubated and stimulated in oxygenated (O_2:CO_2, 19:1) Krebs buffer (37°C) containing 1μM flurbiprofen. Ins(1,4,5)P_3 mass and cGMP content were measured in neutralized trichloroacetic acid extracts using radioreceptor and radioimmunoassays respectively. Changes in tension were measured isometrically in BTSM strips (Grass FT03.c force-displacement transducers, Grass 79D polygraph). Soluble PDE-isoenzymes were separated by anion-exchange chromatography using Q-sepharose. Three peaks of PDE activity were identified (types I(a), II, IV; Reeves et al. 1987) and their sensitivity to inhibition by zaprinast determined according to Thompson & Appleman (1971).
MCh (10μM, EC_{72} for Ins(1,4,5)P_3 response) caused a rapid, transient increase in Ins(1,4,5)P_3 accumulation and a slower, sustained increase in cGMP levels (Table 1). Pre-incubation (20 min) of slices with 10μM zaprinast failed to influence either the MCh-stimulated increase in Ins(1,4,5)P_3 or cGMP. Likewise, incubation of BTSM strips for 20 min with 10μM zaprinast failed to influence the contractile response to MCh (Table 1). The effectiveness of zaprinast to inhibit cGMP hydrolysis was confirmed by its selectivity in reducing PDE-type Ia activity (IC_{50} 0.8, 67, 237 μM for type Ia, II, and IV respectively).

Table 1	Ins(1,4,5)P_3 (pmol/mg prot.)					cGMP (fmol/mg prot.)					Tension (T)	
MCh incubation time(s)	0	5	10	15	60	0	5	10	30	60	pD_2	T_{max} (mN)
+ vehicle	10.1	17.9	14.7	11.9	8.6	105	141	207	453	453	6.45	365
+ zaprinast	9.8	17.4	14.9	12.5	8.1	94	144	239	488	553	6.44	322

Means of triplicate determinations in 3 separate experiments, SEMs <15% for all values.
These data demonstrate that zaprinast does not influence MCh-stimulated Ins(1,4,5)P_3 or cGMP accumulation in BTSM slices, despite potent inhibition of isolated type Ia PDE activity. The previous contrary data in guinea-pig tracheal rings may indicate cGMP regulation of Ins(1,4,5)P_3 accumulation in contaminating non-muscle cells.

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365P EVIDENCE FOR AN EFFECT OF METHOCTRAMINE ON GANGLIONIC NICOTINIC CHOLINOCEPTORS IN THE GUINEA-PIG TRACHEA *IN VITRO*

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Methoctramine has been reported to be a selective antagonist for muscarinic M_2 cholinoceptors in the heart, but has low potency for M_3 muscarinic receptors on smooth muscle (Melchiorre et al., 1987). In guinea-pigs *in vivo*, this antagonist causes facilitation of airway smooth muscle contraction elicited by pre-ganglionic vagal nerve stimulation, due to blockade of pre-junctional M_2 muscarinic autoreceptors on the cholinergic nerve terminals (Watson et al., 1989). Methoctramine has also been reported to block nicotinic receptors in the frog rectus abdominis muscle (Melchiorre et al., 1987) and to have some degree of M_1 muscarinic antagonist activity (Giraldo et al., 1988). The present experiments were designed to investigate the possibility that methoctramine may affect ganglionic cholinoceptors in the pulmonary vagal nerves. The action of methoctramine was compared on the ganglionic nicotinic, pre-junctional M_2 muscarinic and post-junctional M_3 cholinoceptors in the vagally innervated tracheal tube preparation of the guinea-pig.

The trachea with vagi and recurrent laryngeal nerves intact was removed from Dunkin-Hartley guinea-pigs (250-350g) killed by cervical dislocation. The trachea was secured onto an electrode to form a sealed Krebs' Henseleit buffer filled tube, connected to a pressure transducer (Faulkner et al., 1986). Contractions of the trachealis muscle, elicited by pre-ganglionic stimulation (PGS) or by transmural stimulation (TMS) (30V, 30Hz, 0.2ms, 5s every 40s) were recorded as changes in intraluminal pressure. Indomethacin (5μM) and propranolol (1μM) were present throughout the experiments and hexamethonium (75μM) was present during TMS to eliminate any possibility of ganglionic stimulation.

Both PGS- and TMS-induced contractions of the guinea-pig trachea were facilitated by methoctramine 10^{-7}M (increase of 12% ± 3 and 10% ± 4 respectively). This concentration had no effect on ACh-induced tone changes, suggesting that post-junctional M_3 receptors were not blocked. Concentrations of methoctramine greater than 10^{-6}M caused marked inhibition of PGS-induced contractions (decrease of 25% ± 9 at 10^{-5}M) while TMS-induced contractions were not inhibited (decrease of 1% ± 10 at 10^{-5}M). The inhibition of responses to pre-ganglionic stimulation by methoctramine, must be due to an action at the parasympathetic ganglia. An effect on M_1 muscarinic receptors was excluded, since pirenzepine (10^{-9} to 10^{-7}M) did not effect PGS-induced contractions. Therefore the inhibition is probably due to blockade of ganglionic nicotinic cholinoceptors, since DMPP-induced contractions of tracheal segments (10^{-7} to 10^{-6}M) were inhibited by methoctramine (10^{-6}M). Thus in lung tissue *in vitro*, methoctramine would appear to be an effective antagonist for ganglionic nicotinic cholinoceptors and pre-junctional M_2 muscarinic cholinoceptors. Both of these actions occur at concentrations which have no effect on post-junctional M_3 receptors on airway smooth muscle.

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366P GALLAMINE AS A PHARMACOLOGICAL TOOL TO AID THE IDENTIFICATION OF MUSCARINIC RECEPTOR SUBTYPES

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The interaction of gallamine with the 4 muscarinic receptors that can be identified in receptor binding studies has been investigated. Using methods previously described (Michel et al., 1989), M_1 muscarinic receptors present in rat cortical membranes were labeled using 0.5 nM [^{3}H]pirenzepine whilst 0.1 nM [^{3}H]N-methylscopolamine ([^{3}H]NMS) was used to label M_2, M_3 and M_4 muscarinic receptors present in rat cardiac, rat gland (submaxillary) and NG108-15 cell membranes, respectively (Michel et al., 1989). A 50 mM Tris 0.5 mM EDTA assay buffer (pH 7.4 at 32°C) was used and assays were conducted in a volume of 1.25 ml for 2 hrs before separating bound and free ligand using vacuum filtration. Muscarinic receptors in chicken heart and PC12 cell membranes were also labeled using [^{3}H]NMS under identical conditions.

		pIC_{50} (nH)			
Tissue	Receptor	Gallamine	AF-DX 116	Methoctramine	Pirenzepine
Rat Cortex	M_1	6.4 (0.92)	6.5 (0.87)	7.6 (1.02)	8.3 (0.99)
Rat Heart	M_2	7.4 (0.93)	6.7 (1.04)	8.7 (0.95)	6.6 (1.04)
Rat Gland	M_3	5.4 (0.73)	5.6 (0.89)	6.8 (1.01)	7.0 (0.89)
NG108-15 Cell	M_4	6.0 (0.92)	6.6 (1.03)	8.2 (0.91)	7.2 (0.89)
PC12 Cell	M_4	5.9 (0.89)	6.5 (0.91)	8.0 (0.94)	7.0 (0.96)
Chicken Heart	?	7.0 (0.87)	7.1 (0.94)	8.4 (1.02)	7.9 (0.88)

The data are from 4-7 experiments. The SEM was <10%.

Gallamine was an M_2 receptor selective ligand and was able to differentiate between the M_1, M_2 and M_3 muscarinic receptors with selectivities comparable to or greater than those obtained with methoctramine and AF-DX-116. In addition gallamine was able to differentiate between the M_2 and M_4 muscarinic receptor subtypes (25 fold selectivity) whereas pirenzepine, AF-DX 116 and methoctramine displayed lower selectivities (3-4 fold). Although a previous study (Lazareno & Roberts 1989) indicated similarities between rabbit lung (M_4 receptor) and chicken heart muscarinic receptors gallamine, in this study, was able to differentiate between M_4 and chicken heart muscarinic receptors (10-fold selectivity). These data indicate that gallamine, despite its putative allosteric properties (Stockton et al., 1983), can be used as a tool to aid in pharmacologically defining 4 of the 5 muscarinic receptor subtypes identified to date (Bonner et al., 1988).

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367P EFFECTS OF THE K+ CHANNEL OPENERS CROMAKALIM AND RP 49356 ON THE CYCLIC NUCLEOTIDE CONTENT OF GUINEA-PIG ISOLATED TRACHEALIS MUSCLE

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Cromakalim and RP 49356 relax guinea-pig isolated trachealis (Allen et al.,1986; Brown et al.,1989). Tested against spontaneous tone (present study), these agents evoke relaxation equivalent to 70-80% of that induced by aminophylline (1mM) with -log EC50 values of 6.19±0.03 and 5.74±0.07 (mean ± s.e.mean) respectively. In order to determine whether the actions of these agents involve the intracellular accumulation of cyclic nucleotides, muscle-rich strips of guinea-pig trachea were incubated for 15 min in the absence or presence of various drugs. Frozen tissues were pulverised prior to the extraction and radio-immunoassay of cyclic nucleotides. The results of some of these experiments are presented in Table 1.

Table 1

Treatment	cAMP (pmol/mg protein)	cGMP (pmol/mg protein)	Treatment	cAMP (pmol/mg protein)	cGMP (pmol/mg protein)
Control	12.16±0.86(24)	1.05±0.08(22)	Forskolin		
Cromakalim			160nM	16.26±2.40(3)	1.20±0.18(3)
3 µM	13.50±1.80(10)	0.87±0.22(8)	742nM	84.30±13.7(9)*	1.31±0.22(9)
30 µM	14.91±1.90(10)	1.16±0.16(10)	Theophylline		
75 µM	15.45±2.60(9)	1.14±0.10(10)	1mM	13.00±0.66(5)	0.99±0.13(5)
RP 49356					
3 µM	15.79±1.65(5)	1.07±0.06(5)	Sodium nitrite		
30 µM	13.36±2.50(5)	1.12±0.05(4)	2.5mM	8.70±0.91(9)	1.00±0.07(7)
75 µM	12.54±0.92(5)	1.22±0.19(5)	25mM	14.02±1.10(15)	2.09±0.21(15)*

Data indicate mean ± s.e. mean. Figures in parentheses indicate no. of experiments. * indicates a significant increase compared with controls.

Theophylline (1mM) potentiated forskolin in increasing cAMP content. This action of theophylline was not shared by cromakalim (30µM) or RP 49356 (30µM). We conclude that, as for the action of cromakalim in the bovine retractor penis muscle (Gillespie & Sheng, 1988), the relaxant actions of cromakalim and RP49356 in guinea-pig trachea do not depend on the intracellular accumulation of cyclic nucleotides. Cromakalim and RP49356 probably do not share the ability of theophylline to inhibit cAMP-dependent phosphodiesterase.

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368P EFFECTS OF BRL 38227 ON CALCIUM UPTAKE BY INTRACELLULAR STORES IN CULTURED RABBIT AIRWAY SMOOTH MUSCLE CELLS

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The potassium channel activator cromakalim (BRL 34915) is well established as a potent relaxing agent of airway smooth muscle (Allen et al, 1986), but the exact mechanism of action is unclear. Although the primary site of action of this class of compounds is thought to be at plasmalemmal ATP-sensitive potassium channels, it has recently been suggested that in rabbit aorta cromakalim can inhibit Ca^{2+} loading of the noradrenaline-sensitive Ca^{2+} store (Bray et al, 1988). We have investigated the effect of the active enantiomer of cromakalim, BRL 38227, on Ca^{2+} uptake by the intracellular stores, and also on $InsP_3$ and GTPγS induced Ca^{2+} release in cultured airway smooth muscle from rabbit trachealis.

Ca^{2+} loading of the intracellular store was quantified by analysis of ^{45}Ca efflux in digitonin permeabilised monolayers of cells. The cells were preincubated with 10µM BRL 38227 in a solution for permeabilised cells containing 0.1mM EGTA (pH 6.8) for 15 min. A Ca^{2+} loading solution was then applied, which contained in addition 1µM [Ca^{2+}] labelled with ^{45}Ca. A minimum of 10 min preincubation was required for maximal effects to be seen. BRL 38227 reduced the Ca^{2+} content of the intracellular store by 26.6% ± 4.4 (mean ± SD), and slowed the initial rate of Ca^{2+} uptake. Further experiments were performed to investigate the effects of BRL 38227 on $InsP_3$ and GTPγS induced Ca^{2+} release. Preincubation with BRL 38227 during the ^{45}Ca loading period reduced the maximally effective $InsP_3$ (10µM) induced Ca^{2+} release by 21.7% ± 6.9, but had no effect on GTPγS (100µM) induced Ca^{2+} release (n=5).

These results show that BRL 38227 affects Ca^{2+} uptake by the intracellular store in airway smooth muscle. The demonstration that $InsP_3$ induced Ca^{2+} release was reduced, whereas GTPγS induced Ca^{2+} release was unaffected by BRL 38227 implies that $InsP_3$ and GTPγS act on different intracellular stores, and that Ca^{2+} loading of the GTPγS sensitive store possibly involves different mechanisms to that of the $InsP_3$ sensitive store. The mechanism by which BRL 38227 acts on the intracellular store requires clarification, but may involve a direct effect on the potassium channels of the sarcoplasmic reticulum.

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369P EFFECTS OF LEMAKALIM (BRL 38227) AND SALBUTAMOL ON HYPERRESPONSIVE LUNG PARENCHYMA

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Airways hyperresponsiveness and blood eosinophilia are two characteristics of asthma which can be induced in rats by the intravenous injection of Sephadex particles (Taylor and Spicer, 1990). In the present study we have investigated the effects of lemakalim and salbutamol on the hyperreactivity to 5-HT and carbachol observed in vitro in lung parenchymal strips from Sephadex-treated rats.

The parenchymal strips from Sephadex-treated rats were more responsive (and for carbachol, were more sensitive) than those from naive animals (n=8).

	Sephadex-treated		Naive	
	maximum response (mg) ± SEM	EC_{50}(μM) ±95% confidence limits	maximum response (mg) ± SEM	EC_{50}(μM) ±95% confidence limits
5-HT	340±33	4.2 (3.9-4.7)	111±12	3.9 (3.2-4.8)
Carbachol	268±24	2.6 (2.2-3.0)	173±20	6.1 (5.5-6.8)

Lemakalim (10^{-7}-10^{-5}M) or salbutamol (10^{-8}-10^{-6}M) induced relaxations of resting tone in tissues from Sephadex-treated rats (maximal reductions in tension of 162±50mg and 116±28mg respectively) but had no effect in control tissues. In the continuing presence of lemakalim or salbutamol, the subsequent responses to carbachol were markedly inhibited in the parenchymal strips from Sephadex-treated rats so that the responses were similar to those of control tissues. Both lemakalim and salbutamol had little effect on carbachol-induced responses in control tissues. In contrast, against 5-HT-induced contractions, lemakalim (10^{-7}-10^{-5}M) had no significant effect in tissues from Sephadex-treated animals but reduced responses in control tissues. Salbutamol only had a minimal effect, inducing a reduction in the maximum response to 5-HT in tissues from Sephadex-treated rats at the highest concentration (10^{-6}M).

These results suggest that the hyperresponsiveness to carbachol is a voltage-dependent phenomenon since it is inhibited by lemakalim, a potassium channel activator (Arch et al, 1988). As salbutamol also inhibited the carbachol-induced contractions and carbachol has been shown not to contract arterioles (Taylor and Spicer, 1990), it is suggested that carbachol-induced contractions are mainly an airway component. The lack of effect of lemakalim against 5HT-induced contractions suggests these occur by a different (voltage-independent?) mechanism, and probably have a much greater vascular component.

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370P EFFECTS OF 5-HT AND CARBACHOL ON SMALL AND LARGE PULMONARY ARTERIES FROM HYPERRESPONSIVE AND NORMAL RATS.

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Sephadex beads injected into rats induce a blood and lung eosinophilia accompanied by an airways hypersensitivity in vivo and hyperreactivity of lung parenchyma in vitro (Spicer et al, 1989). Sinceparenchymal tissue comprises both airways and vascular smooth muscle, either of these components may contribute to the contractions observed (Clayton et al, 1981). In order to investigate whether the arterial vascular tissue contributes to the hyperreactivity, we have investigated the effects of 5-HT and carbachol on pulmonary arteries, using the myograph apparatus developed by Mulvaney and Halpern (1977).

Male Sprague-Dawley rats (250-350g) were given 1ml of Sephadex (G200) beads in saline (0.5mgml^{-1}) into the hind foot vein on days 0,2 and 5. On days 6 and 7, blood samples were taken from the tail vein, the lungs removed and both total and differential blood counts measured. Small (<300μm) and large (>700μm) pulmonary arteries were dissected out and set up under an intraluminal pressure of 30mmHg according to the method of Leach et al (1989). Naive rats were untreated.

Rats injected with Sephadex beads developed a blood eosinophilia (range 1.0-1.9 x 10^6 cells ml^{-1}; normal values 0.05-0.2 x 10^6 cells ml^{-1}). 5-HT (10^{-7}-10^{-4}M) induced concentration-dependent contractions of the pulmonary arteries from both naive and Sephadex-treated animals. The small arteries from the Sephadex-treated rats exhibited a significantly ($p<0.05$) greater maximal response than those of a similar size from naive animals [Sephadex: 1.35±0.28mN mm^{-1};naive:0.42±0.09mN mm^{-1} (n=8)]. For the large arteries the reverse was true [Sephadex: 0.44±0.05mN mm^{-1};naive: 0.76±0.13mN mm^{-1} (n=8) $p<0.05$]. Carbachol did not induce contractions or relaxations of resting tension in either small or large arteries from Sephadex-treated or naive animals. When tension was induced in the tissues by 5-HT (10^{-4}M), carbachol induced concentration-dependent relaxations in the large but not the small arteries. There was no difference in the relaxations in the large arteries from naive or Sephadex-treated animals (Sephadex: 70±9%; naive: 79±8% - calculated as % of 5-HT-induced maximum contraction, $p<0.05$).

These results suggest that in rat lung parenchyma, 5-HT-induced contractions have a vascular component and hyperreactivity of small arterioles may contribute to parenchymal hyperresponsiveness. Carbachol-induced contractions may be largely airways dependent (Ward et al., 1990), although an effect of carbachol on veins and other cell types present in parenchyma cannot be excluded.

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371P LIMITATIONS OF THE CO-AXIAL BIOASSAY

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Co-axial bioassay (CAB) has been used to establish the existence of a novel smooth muscle relaxing factor from airway epithelium. However some of the relaxing activity may be an artefact due to flaws in the method. We investigated one CAB in which the rat aorta (RA) relaxes when surrounded by a guinea-pig trachea (GPT) (Fernandes et al 1989).

Endothelium-denuded strips of RA (male rats 250-400g) were suspended under 1g initial tension in Krebs-Henseleit buffer aerated with 95%O_2/5%CO_2 (KH) and pre-contracted with 10μM phenylephrine (PE). The strips were surrounded by half-GPTs (from male guinea-pigs, 400-600g) some of which had their epithelium removed. Other GPTs were slit longitudinally to prevent changes in internal diameter. Alternatively, the pre-contracted strips alone were suspended in KH and the O_2 tension (pO_2) lowered using helium gas. The pO_2 was measured using a Neocath 1000 O_2 electrode (Biomedical Sensors). pO_2 was also measured inside half-GPTs suspended in normal KH.

Acetylcholine (ACh, 1-100μM) caused an epithelium-dependent relaxation of the RA surrounded by GPT, a response not seen if the GPT had been slit. The pre-contracted RA maintained its original response to PE for up to 4 hrs in KH with a pO_2 greater than 150mmHg. Below this value, the muscle tension decreased; at 50mmHg all preparations had relaxed by more than 50%. The pO_2 inside the epithelium-intact GPT (209 ± 35mmHg) was significantly lower than outside (550 ± 9mmHg, $p<0.05$), a reduction attenuated by epithelial removal. ACh reduced the internal pO_2 of the epithelium-intact GPT, but had little or no effect in epithelium-denuded or slit GPT (Table 1).

Table 1

		control	1μM ACh	10μM ACh
pO_2 inside GPT (mmHg)	+ epithelium	209 ± 35	116 ± 29	37 ± 15
	+ epithelium, slit GPT	356 ± 41	411 ± 40	435 ± 41
	- epithelium	368 ± 17	362 ± 17	314 ± 22

The changes in pO_2 observed inside the epithelium-intact GPT may contribute towards the relaxation of the RA. Any agonist which causes constriction of airway smooth muscle will reduce the volume and hence the amount of oxygen available inside the airway tube. Indeed, should a factor be generated by airway epithelium, no distinction could be made between a stimulated release and an increase in its local concentration due to a reduction in tracheal volume. These factors should be considered when interpreting results from any CAB.

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372P EOSINOPHIL ACTIVATION ELICITS AN ENHANCEMENT IN THE PERMEABILITY OF THE BRONCHIAL MUCOSA TO ALBUMIN

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In normal airways there is only a limited and controlled transepithelial flux of plasma proteins. In contrast, sustained transepithelial leakage of proteins precedes and is associated with bronchial asthma (Lam *et al.*, 1986; Fabbri *et al.*, 1987). Although the mechanism of this enhanced leakiness is not known, it possibly results from the combined effects of sub-epithelial oedema and leucocyte-induced epithelial injury. We have used an in vitro method to study the effect of eosinophil activation on the permeability of the bronchial mucosa to bovine serum albumin (BSA).

Sheets of airway mucosa were carefully dissected from the trachea and bronchi of bovine lungs. Tissues were mounted between two halves of a chamber containing Eagle's MEM at 37°C. Eosinophils obtained by peritoneal lavage of guinea pigs which had been treated with polymyxin-B were purified (90 ± 1%) by density gradient centrifugation and added to the basolateral side of the tissues (3-3.5 x 10^6 cells in 1ml). The eosinophils were activated with either 5μM A23187 or by opsonization of the epithelium with guinea pig anti-bovine airway mucosa serum. After exposure to the epithelium for 60-180 min the eosinophils were removed, the chambers filled with fresh medium and the permeability of the epithelium determined by measurement of the net unidirectional (basolateral-apical) flux of ^{125}I BSA over 180 min.

In the bronchial mucosa, eosinophils activated by A23187 caused an increase in the unidirectional flux of BSA from 2.5 ± 0.2 to 5.8 ± 0.9 fmol $cm^{-2}min^{-1}$ after exposure to tissues for 60 min (n=4, $P<0.05$). Control experiments showed that A23187 had no intrinsic effect on BSA flux when added to the chambers in the absence of eosinophils. Exposure of eosinophils to opsonized epithelium also elicited an increase in BSA flux from 1.8 ± 0.2 to 4.1 ± 0.3 fmol $cm^{-2}min^{-1}$ (n=5, $P<0.05$). Increasing the exposure time to 180 min had little effect on the enhancement of BSA flux caused by eosinophils activated with A23187 (control 2.9 ± 0.5 versus 5.7 ± 1.1 with A23187). However, the effect of IgG activated eosinophils was slightly greater (control 2.3 ± 0.3 versus 6.1 ± 1.1 with activated eosinophils). None of the above manipulations produced significant changes in the net unidirectional flux of BSA in tracheal preparations.

These observations suggest that eosinophils are capable of producing a rapid increase in the functional permeability of the bronchial, but not the tracheal, epithelium. This phenomenon may account for the increase in protein permeability seen in the airways before and during late phase responses in asthma.

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373P MODULATION OF THE EOSINOPHIL-INDUCED ENHANCEMENT OF BRONCHIAL EPITHELIAL PERMEABILITY

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We have reported that activation of guinea pig eosinophils elicits an increase in the permeability of bovine bronchial epithelium to bovine serum albumin (BSA)(Herbert *et al.*, This Meeting). Histological examination of tissues exposed to eosinophils in this way indicates the presence of focal damage at sites of eosinophil contact (Herbert *et al.*, unpublished observations). We now report experiments which were designed to further characterize the nature of this interaction between eosinophils and the airways epithelium.

Tissues were obtained and mounted in heated chambers as previously described (Herbert *et al.*, This Meeting). Eosinophils were also processed and activated with A23187 as described for 60 min. Net basolateral-apical fluxes of BSA were then measured for 180 min. In the presence of unstimulated eosinophils the control flux of BSA was 2.3 ± 0.2 fmol $cm^{-2}min^{-1}$ (n=12). Addition of the 5-lipoxygenase inhibitor AA-861 (10μM) did not significantly affect this rate, giving a value of 1.8 ± 0.4. Stimulation of the eosinophils with 5μM A23187 resulted in a significant increase in BSA flux, and this was unaffected by the presence of AA-861 added 4 min prior to cell activation (fluxes: 4.1 ± 0.7 without inhibitor, versus 4.0 ± 0.4 fmol $cm^{-2}min^{-1}$, n=4). In the presence of heparin (1000 U ml^{-1}) or α-2-macroglobulin (100 μg ml^{-1}) the control fluxes were 1.6 ± 0.2 and 1.3 ± 0.1 fmol $cm^{-2}min^{-1}$. Eosinophils activated by A23187 in the presence of heparin produced a slightly larger effect on permeability, with a flux of 3.8 ± 0.6 fmol $cm^{-2}min^{-1}$ compared to a heparin-free value of 2.4 ± 0.3 fmol $cm^{-2}min^{-1}$ ($P<0.05$, n=6). In contrast, α-2-macroglobulin had an inhibitory effect with a measured flux of 1.7 ± 0.2 fmol $cm^{-2}min^{-1}$ under these conditions ($P<0.05$ with respect to A23187 activated eosinophils, n=6).

In 4 further experiments, the basolateral side of the epithelium was masked from the eosinophils by placing a 0.45 μm Millipore filter over the tissue. The fluxes of BSA measured after exposing the membranes to unstimulated eosinophils were 2.4 ± 0.4 (filter absent) and 2.9 ± 0.3 (filter present) fmol $cm^{-2}min^{-1}$. In the absence of the filter the BSA flux rose to 7.1 ± 1.8 fmol $cm^{-2}min^{-1}$ after activation with A23187 ($P<0.05$). In contrast, the corresponding measurement of flux made in filter-masked tissues was 2.4 ± 0.2 fmol $cm^{-2}min^{-1}$.

These experiments suggest that leukotrienes are not directly involved in these permeability changes. However, the ability of the broad-spectrum antiproteinase α-2-macroglobulin to inhibit the phenomenon, together with the proximity-dependency of the interaction, suggests that pericellular proteolysis may be one mechanism accounting for this epithelial injury.

Herbert, C.A., Edwards, D., Boot, J.R. & Robinson, C. (1990) This meeting.

374P RECEPTOR-MEDIATED ACCUMULATION OF D-MYO-INOSITOL (1,4,5,) TRISPHOSPHATE MASS IN GUINEA-PIG EOSINOPHILS

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PAF increases the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) in guinea-pig eosinophils by mobilisation of Ca^{2+} from both intracellular stores and from the extracellular compartment (Kroegel *et al*, 1989a,b). Preliminary studies have revealed that PAF stimulates, in both a time- and concentration-dependent manner, the incorporation of [^{3}H] *myo*-inositol into membrane lipids (Kroegel *et al*, 1989c). We now present data in which these studies have been extended to determine if PAF and other agonists also increase D-*myo*-inositol (1,4,5) trisphosphate ($InsP_3$) mass in these cells and whether there is any relationship between $InsP_3$ accumulation and Ca^{2+}-mobilisation.

Eosinophils were harvested from the peritoneal cavity of human serum-treated male guinea-pigs and purified on discontinuous Percoll gradients. Cells (7 x 10^6 per assay; purity > 96%, viability > 99%) were resuspended in HEPES-buffered physiological salt solution containing 1.6 mM $CaCl_2$, equilibrated at 37°C (5 min) and then stimulated with either vehicle, PAF, LTB_4, C5a, PMA or fMLP. $InsP_3$ mass was subsequently determined using a radioreceptor assay (Challiss *et al*, 1988). The $[Ca^{2+}]_i$ was estimated fluorimetrically using fura-2 as described previously (Kroegel *et al*, 1989b).

Challenge of guinea-pig eosinophils with PAF (1 µM for 5 s) increased $InsP_3$ mass 3.7-fold from a resting level of 0.37 ± 0.01 (n = 6) to 1.37 ± 0.23 pmol 10^7 $cells^{-1}$ (n = 6). This effect of PAF was concentration-dependent (mean EC_{50}: 9.6 nM, n = 6) and was abolished in cells pre-treated with the PAF antagonist, WEB 2086 (10 µM for 5 min). Temporally, the accumulation of $InsP_3$ was transient, peaking 5 s post-PAF challenge, and returned to resting levels within 60 s. In addition to PAF, LTB_4 (1 µM), C5a (100 nM) and fMLP (100 µM) also promoted the accumulation of $InsP_3$ in guinea-pig eosinophils to 1.77 ± 0.07 (n = 6), 0.80 ± 0.07 (n = 6) and 0.57 ± 0.01 pmol 10^7 $cells^{-1}$ (n = 6) respectively 5 s post-drug challenge. The tumour-promoting phorbol diester, PMA (1 uM), did not stimulate $InsP_3$ accumulation in these cells. Over the same concentration range, PAF also increased the $[Ca^{2+}]_i$ 3.9-fold (mean EC_{50}: 17.3 nM, n = 6) from 108.4 ± 32.5 nM (n = 32) at rest to 420.8 ± 89 nM (n = 6) after 1 µM PAF. This increase in the $[Ca^{2+}]_i$ in response to 1 µM PAF was transient, the peak effect occurring 10 s after challenge, and returned to basal levels within 60 s. Hence, the peak $InsP_3$ response appeared to precede the peak rise in the $[Ca^{2+}]_i$. LTB_4 (1 µM) and fMLP (100 µM) also elevated $[Ca^{2+}]_i$ to 444.4 ± 72 nM (n = 6) and 206.4 ± 51 nM (n = 6) respectively. The magnitude of the rise in $[Ca^{2+}]_i$ induced by PAF, LTB_4 and fMLP correlated closely with their ability to increase the concentration of $InsP_3$.

In conclusion, the results presented in this study suggest that PAF and other agonists promote the receptor-mediated hydrolysis of phosphatidylinositol (4,5)-bisphosphate in guinea-pig eosinophils with the resultant formation of $InsP_3$. The close temporal relationship between $InsP_3$ accumulation and the increase in the $[Ca^{2+}]_i$ is consistent with a second messenger role for this inositol polyphosphate in releasing Ca^{2+} from intracellular stores in these cells.

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375P INDUCTION OF THROMBOXANE AND E-SERIES PROSTAGLANDIN BIOSYNTHESIS IN GUINEA-PIG EOSINOPHILS STIMULATED WITH PLATELET ACTIVATING FACTOR

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PAF stimulates the metabolism of arachidonic acid by 5'-lipoxygenase in both guinea-pig and human eosinophils to LTB_4 and LTC_4 respectively (Bruynzeel *et al*, 1986; Sun *et al*, 1989). It is not yet known, however, whether PAF can also promote the production of cyclo-oxygenase products in these cells. This is an important point to establish since in other tissues many of the actions of PAF are mediated indirectly via the synthesis and subsequent release of prostaglandins and thromboxane (e.g. Burhop *et al*, 1986; Kawaguchi & Yasuda, 1986). In view of the lack of information regarding the cyclo-oxygenase pathway in eosinophils, experiments were designed to determine whether PAF stimulates prostanoid formation in these cells and, in that event, what contribution these products make to PAF-induced eosinophil activation.

Eosinophils were harvested from the peritoneal cavity of human serum-treated male guinea-pigs and purified on discontinuous Percoll gradients. Cells (purity > 96%; viability > 99%) were resuspended in HEPES-buffered physiological salt solution containing 1.6 mM $CaCl_2$, equilibrated at 37°C (5 min) and then challenged (10 min) with either vehicle, PAF, fMLP, C5a or calcimycin. TXB_2 and PGE_1/E_2 released in to the bathing buffer were measured by RIA. Superoxide anion generation and intracellular Ca^{2+} mobilisation were used as indices of cell activation and were measured using well established methods.

PAF (100 nM for 10 min) stimulated the biosynthesis and release of immunoreactive TXB_2 (from 2.34 $\pm$ 1.02 to 10.4 $\pm$ 2.3 pmol 10^6 $cells^{-1}$, n = 9) and PGE_1/E_2 (from 62.1 $\pm$ 15.8 to 334.2 $\pm$ 47.6 fmol 10^6 $cells^{-1}$, n = 9) from guinea-pig eosinophils. This effect of PAF was concentration-dependent (EC_{50} values: TXB_2: 35.7 $\pm$ 10.9 nM, n = 9; PGE_1/E_2: 19.3 $\pm$ 9.3 nM, n = 9) with maximum stimulation (~ 4.5-fold) of prostanoid synthesis occurring at 1 µM PAF. The ability of PAF to generate TXA_2 was rapid ($t_{1/2}$ = 9.2 $\pm$ 0.9 s), transient (~ 40 s), non-cytotoxic and *non*-competitively antagonised by WEB 2086 (15, 45 and 300 nM for 5 min). On an equimolar basis (100 nM), PAF was significantly more effective than C5a (2.1-fold) and fMLP (3-fold) at stimulating TXB_2 release but much less potent than and calcimycin (0.09-fold). Pre-treatment of eosinophils with the cyclo-oxygenase inhibitor, flurbiprofen (8 µM for 5 min), abolished the ability of PAF to stimulate both TXA_2 and PGE_1/E_2 biosynthesis. Likewise, dazmegrel (50 µM for 5 min), a selective inhibitor of thromboxane synthetase, abolished PAF-stimulated TXB_2 release but markedly augmented (~ 5-fold) the release of PGE_1/E_2. Flurbiprofen pre-treatment did not affect the ability of PAF to elevate $[Ca^{2+}]_i$ or to generate reactive oxygen radicals at any PAF concentration examined.

It is concluded that activation of eosinophils by PAF is receptor-mediated and independent of the concomitant generation of TXA_2. Moreover, the data obtained in flurbiprofen-treated cells also suggest that the release of E-series prostaglandins by PAF does not exert a tonic inhibition of either of the functional responses examined.

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376P LEUKOTRIENE B_4 STIMULATES HYDROGEN PEROXIDE GENERATION IN GUINEA-PIG EOSINOPHILS: POSSIBLE ROLE OF PROTEIN KINASE C

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Leukotriene B_4 (LTB_4) is a potent inflammatory mediator with a variety of pathophysiological activities including stimulation of free radical production in human neutrophils. Recent evidence suggests that this may be mediated by the activation of the Ca^{2+}-activated, phospholipid-dependent protein kinase (C-kinase). We report here results from studies designed to investigate if LTB_4 also stimulates free radical production in eosinophils and the possible involvement of protein kinase C.

Eosinophils were harvested from the peritoneal cavity of human serum-treated male guinea-pigs and purified on discontinuous Percoll gradients and the eosinophils were recovered from the 1.080/1.085 g ml^{-1} and 1.085/1.090 g ml^{-1} interfaces. Cells (5 x 10^5 per incubate, 93% purity; > 99% viability) were resuspended in HEPES-buffered physiological salt solution containing 1 mM $CaCl_2$ equilibrated at 37°C (3 min) and then challenged with either vehicle, leukotriene B_4 (LTB_4) or 4*B*-phorbol-12,13-dibutyrate (4*B*-PDBu). Hydrogen peroxide generation was measured fluorimetrically by horse-radish peroxidase catalysed oxidation of scopoletin (Root *et al*, 1975).

LTB_4 (1 nM - 10 µM) stimulated a prompt and transient generation over basal (1.2 $\pm$ 0.6 nmol 10^6 $cells^{-1}$) of hydrogen peroxide from guinea-pig eosinophils. Both the magnitude and rate of production were concentration-dependent (EC_{50}: rate, 60.2 $\pm$ 17.3 nM, n = 9: magnitude, 70.2 $\pm$ 15.4 nM, n = 9) with maximum stimulation (11.6 $\pm$ 2.4 nmol 10^6 $cells^{-1}$, n = 9) occurring at 10 µM LTB_4. In contrast, the phorbol diester, 4*B*-PDDu (1 nM - 10 µM), effected a sustained, concentration-dependent, production of hydrogen peroxide which appeared to continue for as long as substrate was available. On an molar basis 4*B*-PDBu was more potent (EC_{50}, rate 10.8 $\pm$ 9.0 nM, n = 9) than LTB_4. Kinetically, the effects of LTB_4 were rapid with a maximum rate of production being achieved at 62 $\pm$ 8 s (n = 18) irrespective of the concentration of LTB_4 employed. In contrast, the time-to-peak rate of peroxide generation was inversely related to the concentration of 4*B*-PDBu. Pre-incubation of cells with the novel inhibitor of C-kinase, 1-*O*-hexadecyl-2-*O*-methylglycerol (AMG-C_{16}; 300 µM for 5 min; Kramer *et al*, 1989) antagonised, in competitive manner, the ability of both LTB_4 and 4*B*-PDBu to induce peroxide formation (EC_{50}, LTB_4: rate 94.0 $\pm$ 14.2 nM, n = 4: magnitude, 266 $\pm$ 27.1 nM, n = 4: 4*B*-PDBu: rate 30.4 $\pm$ 5.8 nM, n = 3). Thus, the concentration-response curves were shifted to the right without reduction in the maximum response obtained (concentration ratios: LTB_4 rate 1.56; magnitude 3.78; 4*B*-PDBu, rate 2.8).

It is concluded that the ability of LTB_4 to promote hydrogen peroxide generation in guinea-pig eosinophils may involve, at least in part, the activation of protein kinase C. These results are thus consistent with the proposed mechanisms regulating respiratory burst activity in human neutrophils.

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377P SELECTIVE DESTRUCTION OF A SUB-POPULATION OF CORTICAL NEURONES BY SUICIDE TRANSPORT OF VOLKENSIN, A LECTIN FROM *ADENIA VOLKENSII*

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No technique has been developed for selectively destroying pyramidal neurones in the cerebral cortex of laboratory animals. Hence hypotheses about the possible role of these cells in neuropsychiatric disorders and location of affected receptors (e.g.Middlemiss et al, 1986) are difficult to investigate. Recently, volkensin has been shown to be taken up by nerve terminals and retrogradely transported following injections into the CNS, causing the death of neurones projecting to the injection site (Wiley & Stirpe, 1988). We have extended this to an investigation of projections from layer V of the cortex to the striatum.

Male Sprague-Dawley rats (200-250g) were anaesthetized (fluanisone and midazolam). Volkensin (gift from Prof. F. Stirpe) injections (1µl;2ng/µl) were stereotaxically made into the striatum at 3 separate sites. 28-34 days later, the animals were transcardially perfused with 0.9% saline & 30% sucrose. The brains were snap frozen (isopentane/dry ice -40^{0}C), sectioned (12 µm) and the number of pyramidal neurones in cortical layers of the frontal and parietal cortex was assessed by Nissl staining, with counting of the cell nuclei using image analysis.

Table 1. Laminar distribution of cortical pyramidal cells (number/field) following a volkensin-induced striatal lesion

	Layer V	Layers I-III
Contralateral to lesion	83.80 ± 11.48	68.40 ± 10.97
Ipsilateral to lesion	61.20 ± 6.18*	66.40 ± 8.56

Values are means ± S.D., n = 5, * $p < 0.01$ (Student's t-test)

There was a significant reduction in the number of large neurones in cortical layer V ipsilateral to the lesion when compared to the contralateral side. No similar reduction was seen in cortical layers I-III (Table 1).

These results provide preliminary evidence that specific sub-populations of pyramidal neurones in the cerebral cortex may be destroyed by striatal injection of volkensin.

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378P EXOGENOUS GLYCINE AND D-SERINE POTENTIATE AN N-METHYL-D-ASPARTATE RECEPTOR-MEDIATED SYNAPTIC POTENTIAL IN MOUSE OLFACTORY CORTEX SLICES

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Activation of the N-methyl-D-aspartate (NMDA) receptor is probably dependent on endogenous glycine binding to a specific strychnine-insensitive site on the receptor complex (Thomson, 1989), although it is unclear whether the site is saturated with glycine, or whether changes in glycine concentrations might modulate NMDA receptor-mediated excitatory postsynaptic potentials.

Slices of mouse olfactory cortex 300µm thick were perfused at room temperature (Pickles & Simmonds, 1976) in solution containing Mg^{2+} (1mM) and picrotoxin (25µM). Surface field potentials evoked on supramaximal stimulation of the lateral olfactory tract were amplified and recorded using conventional techniques.

Glycine (0.01 to 1mM), and its structural analogue D-serine (0.01 to 2.5mM), had no significant effect on the monosynaptically-evoked N'a'-wave, a potential mediated by kainate/quisqualate receptors (Collins & Buckley, 1989), but increased the area of the di/polysynaptic N'b'-wave, which is mediated by NMDA receptors (Collins, 1982), in a concentration-dependent manner: the maximum increases in area (means ± s.e.means, n = 6-8) were 15.7 ± 4.0% for glycine (1mM) and 56.4 ± 7.7 % for D-serine (1mM).

Both indole-2-carboxylate (I-2-C) and 7-chlorokynurenate (7-CK), two antagonists of the glycine regulatory site (Huettner, 1989; Thomson, 1989), reduced the areas of the N'b'-waves, the mean (± s.e.mean, n=6) IC_{50} values being 1.8 ± 0.3mM and 12.8 ± 2.1µM, respectively. D-serine (1mM; glycine not tested) competitively antagonized these effects : for example, the mean (± s.e.mean, n=6) dose ratios for D-serine in the presence of I-2-C and 7-CK were 4.02 ± 0.68 and 6.92 ± 1.0, respectively. In contrast, although DL-2-amino-5-phosphonopentanoate reduced the area of the N'b'-wave in a concentration-dependent manner (IC_{50} = 4.2 ± 0.22µM; mean ± s.e.mean, n = 5), D-serine (1mM) did not antagonize this effect (corresponding dose ratio was 0.998 ± 0.09).

These results show that in isolated mouse olfactory cortex slices, synaptically activated NMDA receptors are subject to modulation by drugs acting at the glycine regulatory site, and that under the present experimental conditions, the site is not saturated with endogenous glycine.

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379P POSSIBLE INTERACTIONS OF 1,2,3,4-TETRAHYDROAMINOACRIDINE WITH N-METHYL-D-ASPARTATE RECEPTORS

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The drug 1,2,3,4-tetrahydroaminoacridine (THA) has been claimed to be useful in the palliative treatment of patients with Alzheimer's disease (Summers et al., 1986). The aim of the present study was to investigate the reported interaction of THA with the N-methyl-D-aspartate (NMDA) receptor complex (Albin et al., 1988).

Olfactory cortex slices 300 μm thick were prepared from adult white mice and perfused with solution containing Mg^{2+} (1mM) in such a way that the d.c. potential across each slice was monitored continuously on a chart recorder (Brown & Galvan, 1979). Low concentrations of THA (0.01 to 0.2mM) potentiated the peak depolarization evoked by a submaximal concentration of NMDA (0.1mM); in one series of experiments, THA (0.01mM) potentiated the depolarization by 55.8 ± 6.7% (mean s.e.mean, n=3). At concentrations above 0.2mM, THA reduced NMDA-evoked responses. In slices perfused with 0.1mM 3,4-diaminopyridine (DAP), all concentrations of THA attenuated NMDA responses, the IC_{50} value being between 0.2 and 1mM (3 slices).

Possible interactions of THA with the glycine regulatory site of the NMDA receptor complex were studied in slices perfused with DAP (0.1mM). D-serine (1mM), an analogue of glycine, partialy reversed the reduction in NMDA (0.1mM) responses caused by THA (0.2mM): mean ± s.e.mean (n=5) percentage reduction by THA alone 50.7 ± 7.5; plus D-serine 27.9 ± 10.2 (significant difference, paired Student's t-test, $P < 0.05$). Similarly, pre-perfusion of slices with D-serine (1mM) partially prevented the THA- (0.2mM) induced reduction in NMDA response : mean ± s.e.mean (n=5) percentage reduction by THA plus D-serine 32.4 ± 5.7; THA alone 48.2 ± 6.0 (significant difference, paired Student's t-test, $P < 0.05$).

These results suggest that the reported ability of THA to potentiate the neurotoxicity of quinolinic acid (Zhu et al., 1988) might result from a block of potassium channels (Roganozki, 1987) and that in addition to interacting with the phencyclidine site of the NMDA receptor complex (Albin et al., 1988), THA might also act as an antagonist at the glycine regulatory site.

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380P EFFECTS OF PCP AND SIGMA LIGANDS ON NMDA- AND DTG-INDUCED CONVULSIONS AND MORTALITY IN MICE

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Little is known about the functional role of PCP/sigma sites identified in the central and peripheral nervous system (Itzhak, 1988). This study was undertaken to determine whether any functional relationship exists between N-methyl-D-aspartic acid (NMDA) and 1,3 di-o-tolylguanidine (DTG), a sigma ligand, in a model of NMDA- or DTG-induced convulsions and mortality in the mouse. Increasing concentrations of NMDA (50-300mg/kg,i.p.) and DTG (50-200 mg/kg, p.o.) were administered to mice (Swiss I.O.P., Iffa Credo, France) to determine the optimum concentration for inducing convulsions and mortality. DTG (120 mg/kg, p.o.) and NMDA (200mg/kg, i.p.) caused convulsions followed about 2 min later by mortality in 90-95% of mice. The effects of competitive (AP7) and non-competitive (PCP, MK801 (+)SKF10,047) NMDA antagonists, sigma ligands (haloperidol, 3PPP) and anticonvulsants (diazepam, phenytoin sodium, phenobarbitone) were evaluated on NMDA- or DTG-induced convulsions or mortality respectively. Unless otherwise stated, drugs were usually administered 30 min before NMDA or DTG.

NMDA-induced convulsions and mortality were reduced by AP7 (ED_{50} = 52mg/kg,s.c.), PCP (ED_{50} = 3mg/kg,s.c.) and (+)MK801 (ED_{50} = 0.4mg/kg,i.p.) and only partially (40%) by (+)SKF 10,047 (50mg/kg,i.p.). However, when administered at the same time as NMDA, SKF 10,047 caused virtually total inhibition between 12.5-50mg/kg,i.p.but none at lower doses (6 and 9 mg/kg,i.p.). However, none of the sigma ligands or anticonvulsant drugs had any effect on NMDA-induced convulsions or mortality. With respect to DTG-induced convulsions or mortality, no inhibitory effects were observed with either NMDA antagonists or sigma ligands or indeed anticonvulsant drugs.

These findings suggest that NMDA- and DTG-induced convulsions and mortality are mediated by independent mechanisms. The observation that the effects of NMDA are only reduced by NMDA antagonists and not by any of the sigma ligands or anticonvulsant drugs suggest that blockade of potassium channels, at least in part, may be implicated. In contrast, it is less clear how DTG exerts its effects. If the effects of DTG are imediated through sigma receptors it may be of a subtype not sensitive to Lthe drugs used in this study.

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381P POTASSIUM THIOCYANATE-INDUCED INCREASES IN EAA RECEPTOR BINDING IN THE RAT VISUALISED BY [^{3}H]-L-GLUTAMATE INVOLVES NMDA RECEPTORS IN ADDITION TO AMPA RECEPTORS

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Potassium thiocyanate (KSCN) (100 mM) has been reported to increase [^{3}H]-AMPA (Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) binding to AMPA-sensitive excitatory amino acid (EAA) receptors in brain homogenate preparations (Honore et al., 1985). We have investigated the effects of KSCN on the binding of [^{3}H]-L-glutamate to EAA receptors in brain sections. Brain sections (20 μm) from male Sprague-Dawley rats were cut on a cryostat and thaw-mounted onto subbed slides. Sections were lyophilised and washed in ice-cold Tris acetate buffer (50 mM, pH 7.2) for 120 minutes. The sections were lyophilised then incubated in 100 nM [^{3}H]-L-glutamate (48 Ci/mmol) in Tris acetate (50 mM, pH 7.2) for 30 minutes at 25°C. The incubation was terminated and unbound radioligand separated from free by washing for 30 seconds in Tris acetate (4°C) and a dip wash in acetone/2.5% gluteraldehyde. Sections were immediately dried in a stream of cold air. N-methyl-D-aspartate (NMDA) receptor binding was defined as that displaced by 100μM 3-((+)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), binding to AMPA-sensitive sites was displaced by 5 μM AMPA. Experiments were conducted in triplicate, and data are presented on studies in four animals.When KSCN (100mM) was added to the incubation medium, it significantly increased all aspects of glutamate binding (matched pair t test - one tailed).

Table 1. Increases seen in glutamate binding with KSCN.

	mean % increase with KSCN (100mM)	s.e. mean
Total Bound Glutamate	67.5	± 15.3
CPP sensitive	67.5	± 26
AMPA sensitive	763	± 310

The CPP-sensitive [^{3}H]-L-glutamate binding sites observed in the presence and absence of KSCN may reflect subtypes of the NMDA receptor. The presence of such NMDA subtypes has been proposed by [^{3}H]-CPP binding (Olverman et al., 1986).

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382P SPERMIDINE ENHANCEMENT OF [^{3}H]-MK-801 BINDING TO THE NMDA RECEPTOR COMPLEX IN HUMAN BRAIN

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Spermidine stimulates binding of [^{3}H]-MK-801 to the N-methyl-D-aspartate (NMDA) receptor complex in rat cortex. This study examined the effects of spermidine in human brain.

Assays were performed in triplicate as described previously (Procter et al., 1989). Grey matter from frozen blocks of posterior frontal cortex of 4 control subjects (age range 81-85 y; post-mortem delay12-48 h; 3 right and 1 left hemisphere; 1 male and 3 females; 2 subjects died suddenly, 2 after protracted illness) was homogenised and washed extensively. Assays were performed in 1ml Tris HCl (5mM, pH 7.4) containing approximately 250μg protein, a final concentration of 5nM [^{3}H]-MK-801 and other agents where appropriate and non-specific binding determined with 10μM MK-801. Except for time course studies incubations at 25°C were terminated after 45 min using a Skatron cell harvester. Spermidine enhanced binding of [^{3}H]-MK-801 to the NMDA receptor complex. Stimulation was dose-dependent with a maximum value of 319 + 71 fmol/mg protein (n=7) at 200μM spermidine (basal 56 ± 5 fmol/mg protein) and an EC50 of 89 ± 22μM (6). Addition of 1μM glutamate and 1μM glycine reduced the EC50 to 5.5±0.7μM (p=0.01, Mann-Whitney U-test). This enhancement reflected an increased rate of association of [^{3}H]-MK-801. The rank order of potency of inhibitors of spermidine stimulated [^{3}H]-MK-801 binding was AP5 > 7-chlorokynurenic acid > ifenprodil (Table 1).

Table 1 The effect of inhibitors on spermidine stimulated [^{3}H]-MK-801 binding.

Drug	Mean IC50 value (μM)	Hill coefficient
AP5	0.5 ± 0.3	1.0 ± 0.06
7-Chlorokynurenic acid	24 ± 19	0.86 ± 0.06
Ifenprodil	91 ± 28	0.78 ± 0.05*

*Indicates significant difference from unity ($p<0.05$); values are mean ± s.e.mean; n=4.

IC50 values and Hill coefficients were estimated using linear regression analysis of Hill plots.

Maximal binding induced by spermidine was significantly reduced in the presence of increasing concentrations of each inhibitor, ($p<0.001$, Student's 't' test), consistent with a non-competitive mode of action.

These data show that spermidine regulates the NMDA receptor complex in human brain and provides further evidence that the NMDA receptor coupled ion-channel in the cortex of human is similar to that in the cortex of rat.

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383P A STUDY OF THE POSSIBLE MECHANISMS BY WHICH POLYMIXIN B SELECTIVELY ANTAGONIZES RESPONSES EVOKED BY N-METHYL-D-ASPARTATE

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Responses evoked by N-methyl-D-aspartate (NMDA) are antagonized by polymyxin B [PB; IC_{50} = 24.3 ± 3.8μM (mean ± s.e.mean, n=5)] whereas responses to kainate and quisqualate are reduced by less than 20% at a concentration of PB of 0.5mM (Collins & Ellis, 1988). The aim of the present experiments was to study possible mechanisms by which PB antagonizes NMDA.

Slices of mouse olfactory cortex 300μm thick were perfused with a Mg^{2+}-free solution at room temperature and the d.c. potential across each slice monitored continuously (Collins & Surtees, 1986). Depolarizations evoked by 1 min applications of NMDA to the pial surface of slices were recorded as negative deflections on a chart recorder and quantified by measuring their peak amplitudes.

In the first series of experiments, the effect of PB (50μM) and the competitive NMDA antagonist DL-2-amino-5-phosphonopentanoate (APP, 50μM) on log concentration-effect curves to NMDA was assessed. Both alone and together, the drugs caused parallel shifts in the NMDA curves to the right, the mean (± s.e.mean, n=4-6) dose ratios being : PB 3.6 ± 0.42; APP 9.6 ± 1.1; PB + APP 31.9 ± 3.7. The value of the latter dose ratio indicates that APP and PB act independently at separate sites on the NMDA receptor complex (Abramson et al., 1969).

The second series of experiments was designed to ascertain whether PB blocked the strychnine-insensitive glycine site of the NMDA receptor complex. Neither glycine nor its structural analogue D-serine (up to 1mM) affected responses to NMDA, neither did they reverse the reduction in NMDA responses by PB (50μM, 5 slices). In contrast, in all 4 slices tested, D-serine (1mM; glycine not tested) completely reversed the reduction in NMDA responses caused by indole-2-carboxylate (2.5mM), a selective antagonist of the glycine regulatory site (Huettner, 1989).

These results suggest that PB does not block either the NMDA recognition site or the glycine regulatory site of the NMDA receptor complex.

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384P BEHAVIOURAL EXCITATION AND NEURONAL LOSS INDUCED BY INTRAHIPPOCAMPAL TETANUS TOXIN IN RATS ARE PREVENTED BY CGP 37849

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Tetanus toxin (TT) is a clostridial neurotoxin which selectively blocks the feed-forward and recurrent GABAergic inhibition in rat CA1 hippocampal pyramids (Calabresi et al., 1989). Recently, we have suggested that the long-lasting disinhibition by unilateral injection of TT into the hippocampus may be responsible for the behavioural excitatory effects and the neuronal loss observed in the CA1 hippocampal area in rats (Bagetta et al., 1990a). In support of this idea we have recently reported that systemic pretreatment with MK801 (0.3 $mgkg^{-1}$), a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, prevents the effects of TT (Bagetta et al., 1990b). To establish whether excitatory transmission is involved we have attempted to antagonize the effects of TT by using a selective and competitive NMDA receptor antagonist, namely CGP 37849 (see Fagg et al., 1989). Adult male Wistar rats (250-280g) were anaesthetized with chloral hydrate (400 $mgkg^{-1}$ i.p.) and TT (1000 mouse minimum lethal doses, MLDs) unilaterally microinjected into the dorsal hippocampus (coordinates: AP = 4.0mm; L = 2.0mm; V = 2.4mm). The volume of injection was 1μl (rate $1\mu lmin^{-1}$). CGP 37849 (0.3, 3.0 or 10 $mgkg^{-1}$ i.p.) was administered one hour before and after TT injection and then, once daily for 10 days. Behavioural activity was studied twice daily and histological examination performed as previously described (Bagetta et al., 1990a). A single injection of TT (1000 MLDs; n= 10 rats) resulted in time-dependent behavioural excitation and lethal effects. The same dose of toxin produced a significant ($p<0.05$; n= 3 rats) neuronal loss in the CA1 pyramidal cell layer 7 and 10 days but not 24 hours after TT injection. No damage was observed in other sectors (CA2, CA3 and dentate gyrus) of the hippocampus. Pretreatment with CGP 37849 (0.3 $mgkg^{-1}$; n= 10 rats) did not show any protection against the behavioural, lethal and neuropathological (n= 3 rats) effects induced by TT. A higher dose (3.0 $mgkg^{-1}$) conferred partial protection. In fact, behavioural excitation was observed in 4 out of 6 animals treated but generalized convulsions were observed only in 2 rats. Neuronal damage was shown in 1 out of 4 rats analyzed 10 days after TT injection. Full protection was conferred by 10 $mgkg^{-1}$ of CGP 37849 (n= 10 rats) and in no instance was any neuronal loss observed. In conclusion, the present results provide further evidence implicating excitatory transmission in the behavioural, lethal and neuropathological effects induced by intrahippocampal injection of TT in rats and suggest also that excitatory amino acid antagonism might be a rational strategy in the treatment of tetanus intoxication in humans.

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385P INJECTIONS OF EAA ANTAGONISTS IN THE ENTOPEDUNCULAR NUCLEUS OF THE RAT ALLEVIATE RESERPINE-INDUCED AKINESIA BY AN ACTION AT NMDA RECEPTORS

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The neural mechanisms underlying parkinsonian akinesia involve overactivity in the excitatory subthalamic input to the medial pallidal segment (this is the primate analogue of the rodent entopeduncular nucleus). EAA antagonists may alleviate akinetic symptoms by antagonising the subthalamic inputs to the entopeduncular nucleus. Previously we reported that in the 6-OHDA rodent model of parkinsonism, the broad spectrum EAA antagonist kynurenate (40 μg) alleviated akinesia when injected into the entopeduncualr nucleus (Brotchie et al., in press). Here we report experiments aimed at defining the pharmacology of this anti-akinetic effect by investigating the dose-response relationships of the EAA antagonists kynurenate (broad spectrum antagonist), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, AMPA receptor selective), 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, NMDA receptor specific) in the reserpine-induced parkinsonian model.

Under general anaesthesia (pentobarbitone, 60 mg/kg) indwelling cannulae were implanted bilaterally to lie above the entopeduncular nucleus in male Sprague-Dawley rats. Akinesia was induced by subcutaneous injection of reserpine (5 mg/kg). EAA antagonists were injected unilaterally in volumes of 500nl. Mobility was assessed by a measure of the distance moved by the forelimb contralateral to the injection in the 15 minutes immediately post-injection. Injections were performed in three sites for each drug concentration. Injection site locations were identified with cresyl violet histology.

No increases in locomotor activity were observed following vehicle injections. Similarly in control injections centred on the ventral thalamus or lateral hypothalamus no anti-akinetic effects were observed. Injection of kynurenate, CNQX and CPP into the entopeduncular nucleus caused a dose dependent reversal of akinesia. The potency order was CPP > kynurenate > CNQX (EC_{50}s 5.4 mg/ml, 20.1 mg/ml and 22.9 mg/ml respectively). The results demonstrate that antagonism of NMDA receptors in the entopeduncular nucleus is capable of reversing akinesia in the catecholamine depleted rat. We suggest that akinesia is underpinned by excessive stimulation of the entopeduncular nucleus by EAAs released by an overactive subthalamic nucleus input. Recently it has been reported that the EAA antagonist MK-801 increases locomotor activity in the reserpine-induced akinetic mouse (Carlson and Carlson, 1989). The data presented here suggest that these systemically adminstered effects of NMDA antagonists may act in part on the entopeduncular nucleus.This report adds weight to the speculation that EAA antagonists, in particular those acting at NMDA receptors, may be of therapeutic value in parkinsonism.

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386P SYNTHESIS AND CHARACTERIZATION OF TWO NOVEL AGONISTS FOR THE KAINATE RECEPTOR

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A large number of ligands specific for the glutamate receptor are known but the purification of glutamate-binding proteins has been hindered by a lack of high affinity agonists or antagonists which would be suitable for affinity chromatography. Here we report the synthesis of two novel glutamate receptor agonists which were designed to be potential ligands for the affinity chromatographic purification of the kainate subtype of the glutamate receptor.

Two synthetic strategies were used to derivatize kainic acid, both involving the derivatization of the isopropenyl group. In the first route the dimethyl ester of N-(butoxycarbonyl)kainic acid was coupled to an aromatic amine in the presence of tert-butyl nitrite and a palladium catalyst (Conway et al., 1984). The m-nitrostyryl derivative of kainic acid so produced was then reduced using sulphurated borohydride and deprotection with trimethylsilyliodide gave 2-carboxy-4-(1-methyl-1-aminostyryl)-3-pyrrolidineacetic acid. The second approach was to epoxidize dimethyl-N-(butoxycarbonyl)kainic acid with m-chloroperbenzoic acid and then open the resulting epoxide with an organocopper reagent generated in situ from 2-(2-bromoethyl)-1,3-dioxolane. Dehydration and deprotection were accomplished in a single step with trimethylsilyliodide to give 2-carboxy-4-(1-methyl-1-pentenal)-3-pyrrolidineacetic acid.

The affinity of these derivatives for the kainate receptor was determined using a [^{3}H]kainate binding assay. Synaptic plasma membranes (0.25-0.35mg) from whole rat brain were incubated in the presence of 5nM [^{3}H]kainate (55.4Ci/mmol) for 30 min at 4°C in 50nM tris-acetate (pH6.8) in a final volume of 0.2ml. Displacing agents were added in various concentrations and non-specific binding determined by addition of a saturating concentration of L-glutamate (0.1nM). Incubations were terminated by dilution and filtration through Whatman GF/C filters. Under these conditions binding of [^{3}H]kainate was reversible and saturable (B_{max}=332fmol/mg) and was to a single population of sites (k_d=10.5nM). Both derivatives displaced kainate from kainate binding sites, the m-aminostyryl derivative with a K_i=728±12nM (S.E. Mean, n=3) and the propenal derivative with a K_i=1364±16nM (S.E. Mean, n=3).

In collaboration with Prof. J.C. Watkins and Dr. P. Pook in Bristol we have measured the ability of the derivatives to depolarize neonatal rat spinal cord in the presence of tetrodotoxin. The depolarizing potencies of the m-aminostyryl derivative and the pentenal derivative were 48-fold less and 200-fold less respectively than that of kainic acid and this correlates with the measured binding affinities. Both of these novel compounds are therefore kainate receptor agonists with potential as purification agents for the kainate receptor and the synthetic routes followed for their production could be exploited for the further production of new kainate derivatives.

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387P ANTICONVULSANT AND ANTIEPILEPTOGENIC ACTIONS OF A STRYCHNINE-INSENSITIVE GLYCINE RECEPTOR ANTAGONIST IN THE KINDLING MODEL OF EPILEPSY

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Electrical kindling of the rat brain is a well-established animal model of epilepsy in which both the development and expression of limbic seizure activity can be studied. Competitive N-methyl-D-aspartate (NMDA) receptor antagonists inhibit the development of kindling and suppress fully kindled seizures in this model (Croucher et al, 1988). 7-Chlorokynurenic acid (7-ClKYN) is a potent and selective antagonist acting at the glycine modulatory site of the NMDA receptor-ion channel complex (Kemp et al, 1988). We report here the powerful anticonvulsant and antiepileptogenic actions of this new class of NMDA antagonist against electrically kindled seizures.

Details of the kindling protocol are described elsewhere (Croucher et al, 1988). Briefly, stainless steel bipolar electrode/guide cannula units were implanted into the right basolateral amygdala of male Sprague-Dawley rats. At least one week after surgery animals were given daily focal injections of drug or buffer alone (controls) followed 30 min later by the kindling stimulus. The development of electroencephalographic and motor seizure responses (graded 0-5) was monitored. Subsequently, in fully kindled animals (3 consecutive Stage 5 seizures), generalized seizure thresholds (GSTs) were estimated using a method of ascending limits. The influence of focally administered glycine receptor ligands on GST, duration of afterdischarge (AD) and severity of motor response was then determined.

In fully kindled animals, 7-ClKYN dose-dependently increased the GST (controls 197.5±20.7µA; 10 nmol 280±34.8 µA, $P<0.05$; 50 nmol 405.0±53.3 µA, $P<0.025$; mean±s.e.mean, n = 5). Co-injection of 7-ClKYN, 10 nmol plus glycine, 40 nmol produced no significant change in GST. Mean control AD (69.7±7.2s) and motor seizure (56.8±7.2s) durations were unaffected by either dose of 7-ClKYN. Focal injection of the glycine agonists, glycine (10 or 50 nmol), D-serine (50 nmol) or D-alanine (50 nmol) failed to influence any of the parameters of the kindled seizure response.

When injected prior to the daily kindling stimulations 7-ClKYN, 10 nmol also significantly inhibited the development of the kindled seizures. After 13 stimulations all control animals (n=6) showed fully developed (Stage 5) seizures. The 7-ClKYN-treated group (n=6) showed a significantly reduced seizure response (mean rating 3.0±0.5; $P<0.01$). Even after 20 stimulations the drug-treated group showed little further progression towards full seizure kindling (mean rating 3.3±0.6; $P<0.01$). Mean AD duration was significantly reduced following many, though not all of the kindling stimulations. No overt behavioural toxicity was evident following any of the drug administrations.

These results demonstrate that antagonism of strychnine-insensitive glycine receptors produces both anticonvulsant and antiepileptogenic effects in the kindling model of epilepsy and adds further evidence for the key involvement of NMDA receptors in generative mechanisms of epilepsy. The lack of effects of glycine agonists may be due to saturation of these sites by the relatively high levels of glycine *in vivo*.

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388P 'STABLE' EXPRESSION OF FUNCTIONAL α_1 AND β_1 SUBUNITS FOR THE VERTEBRATE NEURONAL $GABA_A$ RECEPTOR IN CHINESE HAMSTER OVARY CELLS

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The elucidation of the subunit structure for the vertebrate $GABA_A$ receptor and the cloning and sequencing of the corresponding cDNAs for the subunit proteins has enabled the use of more convenient cells for receptor expression studies. This approach, using mostly the *Xenopus laevis* oocyte translation system, or transient expression in secondary cell lines, requires the injection or transfection of the relevant mRNAs or cDNAs into the cells prior to experimentation. We report here, for the first time, the creation of a 'stable' Chinese hamster ovary cell line (CHO), which has incorporated into the genome, the cDNAs corresponding to the bovine α_1 and β_1 subunits for the $GABA_A$ receptor. CHO cells were initially transfected with an expression vector for both α_1 and β_1 cDNAs using a conventional calcium phosphate method. The transcription of the cDNAs was driven by the mouse mammary tumour virus promoter (MMTV) following induction with dexamethasone. The expression vectors also contained a selection (gpt)-resistance cassette for the establishment of stable cell lines. Cells which were successfully transfected, were selected by using a HAT containing culture medium. Functional $GABA_A$ receptor expression was induced by incubating the CHO cells for 48 hours with 1µM dexamethasone.

By using patch clamp recording techniques on 'induced' CHO cells, the expression of a functional $GABA_A$ receptor was apparent on bath perfusion of GABA (50-100µM). The GABA response was readily enhanced by 10µM pentobarbitone, blocked by 10-15µM bicuculline, but not affected by 5-10µM flurazepam or 10µM chlorazepate. The GABA response reversed at -38mV close to the calculated E_{Cl} under these recording conditions.

Ligand binding studies using ^{3}H-muscimol or ^{35}S-t-butylphosphorothionate (TBPS) revealed approximately 100-200 binding sites per cell. This value was comparable to the 100-150 expressed GABA receptors estimated from electrophysiological studies. The GABA receptor binding was enhanced by 100µM 5β-pregnan-3α-ol-20-one but ^{3}H-flurazepam binding was undetected.

This cell line indicates that the modulatory binding sites for barbiturates, steroids, TBPS and bicuculline all reside on the α and β subunits. Furthermore, CHO cells will provide a useful vehicle for the expression of functional heteromeric and homomeric $GABA_A$ receptors constructed from both 'normal' and mutated protein subunits.

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389P HISTAMINE H_1 ANTAGONISTS MODULATE SEIZURE PATTERN INDUCED BY ELECTROSHOCK IN MICE

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Evidence is now accumulating that histamine may act as an anticonvulsant neurotransmitter in animals and man. We have reported previously that centrally- but not peripherally-acting H1 antihistamines potentiate chemically-induced seizures in mice (Fairbairn & Sturman, 1989).
Female TO mice (Tucks) were pretreated with antihistamine or vehicle i.p. 30 mins before exposure to sine wave shock of 0.5s duration, 100v and variable current, delivered via ear electrodes from a Rodent Shocker (Type 221, Hugo Sachs Elektronik KG). The subsequent clonic and tonic convulsive behaviour was scored for intensity and duration. Initially the threshold current to produce tonic seizures in 50% of the mice (CD50) was determined using an up-down procedure and probit analysis (Finney, 1971). Table 1 shows that centrally- but not peripherally-acting H1 antagonists produced small dose-related increases in CD50 threshold indicating some protection against tonic seizures. However, an increase in incidence and duration of clonic convulsive behaviour was seen in these mice at low current levels. Further experiments confirmed that clonic convulsions were seen infrequently in control or acrivastine-treated mice, at current levels $\leqslant$ 5mA, clearing within 1-2s of shock application. In contrast, diphenhydramine and mepyramine pretreatment produced dose-related prolonged clonic convulsions after a 5mA shock, characterised by hindlimb paddling, jerky limb movements and increased locomotor activity with ataxia (incidence and median duration 66% and 46.4s* at 30mg kg^{-1}, 33% and 42.9s* at 20mg kg^{-1} diphenhydramine; 66% and 42.6s* at 20mg kg^{-1},25% and 23.9s at 10mg kg^{-1} mepyramine respectively. (* = $p < 0.01$, Mann Whitney U test).

Table 1 Threshold currents producing 50% incidence of tonic convulsions (95% confidence intervals) in groups of 30 female TO mice pretreated with antihistamines 30 mins previously.

Pretreatment (dose mg kg^{-1})	CD50 (mA)	Pretreatment (dose mg kg^{-1})	CD50 (mA)
Saline	4.49 (4.93-4.01)	Acrivastine (10)	4.89 (5.60-4.28)
		Acrivastine (1)	4.39 (4.74-4.06)
Diphenhydramine (30)	7.24 (9.01-5.81)	Mepyramine (20)	5.05 (5.83-4.37)
Diphenhydramine (20)	5.97 (6.81-5.23)	Mepyramine (10)	4.71 (5.41-4.10)

This potentiation of clonic but not tonic seizures may indicate histaminergic involvement in seizure spread.

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390P A23187 AND 2-CHLOROADENOSINE INCREASE GUINEA-PIG BRAIN HISTAMINE-INDUCED INOSITOL PHOSPHATES ACCUMULATION BY DIFFERENT MECHANISMS

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We have shown previously that the accumulation of [^{3}H]inositol phosphates ([^{3}H]InsPx) generated by histamine (HA) in mouse cerebral cortical slices is markedly reduced by lowering the ambient calcium ion concentration, and enhanced by the inclusion of the calcium ionophore A23187 in the medium (Alexander et al., 1989). The inhibitory effect of a stable analogue of adenosine (2-chloroadenosine, 2CA) on HA-induced [^{3}H]InsPx accumulation in this tissue was shown not to be infuenced by either of these manipulations and it was therefore suggested not to be mediated by an influence on calcium ion movements (Alexander et al., 1989). In the guinea pig, adenosine analogues potentiate the [^{3}H]InsPx response to HA (Hill & Kendall, 1987). We have therefore investigated whether changes in either extra- or intracellular calcium ion concentration alter the potentiatory response to 2CA in the guinea pig.

[^{3}H]InsPx accumulation in guinea pig cerebral cortical slices (350 x 350 um) was carried out in the presence of 5 mM LiCl and 1 U/ml adenosine deaminase as previously described (Alexander et al., 1989).

As in the mouse, addition of 0.2 mM EGTA in the absence of added calcium ions prevented HA-induced [^{3}H]InsPx accumulation and any potential augmentation of this response by 2CA (n=5). Basal accumulation of [^{3}H]InsPx was not altered by varying the added calcium ion concentration over the range 0 - 1.3 mM. The presence of HA (0.3 mM) raised the accumulation of [^{3}H]InsPx in the absence of added calcium ions to 150$\pm$7 % of basal accumulation (n=5). The response to HA was enhanced in an apparently linear fashion with increasing ambient calcium ion concentration, and at 1.3 mM was 322$\pm$36 % basal (n=5). The presence of 2CA enhanced the response to HA at all concentrations of added calcium ions by 66-97 %.

In a second series of experiments using medium containing 1.3 mM $CaCl_2$, HA (0.3 mM) and HA plus 2CA (33uM) increased [^{3}H]InsPx accumulation from 535$\pm$116 dpm to 1481$\pm$82 dpm and 2189$\pm$84 dpm, respectively (n=3). A23187 at a maximally active concentration (33 uM) induced a 2-fold increase in the accumulation of [^{3}H]InsPx, which was unaffected by the presence of 2CA (33 uM). The response to HA was elevated in the presence of A23187 from 1141$\pm$290 dpm (A23187) to 3000$\pm$636 dpm (A23187 + HA). However, 2CA was still able to further increase the HA response by almost 50 %.

These results indicate that the HA-stimulated [^{3}H]InsPx response of guinea pig cerebral cortical slices is sensitive to manipulations of both extra- and intracellular calcium ion concentrations. Furthermore, the augmentatory response to 2CA appears not to be mediated by alterations in calcium ion movements.

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391P EFFECTS OF A CRUDE BOVINE BRAIN EXTRACT OF CLONIDINE-DISPLACING SUBSTANCE ON [^{3}H]-MYO INOSITOL UPTAKE AND PHOSPHOINOSITIDE HYDROLYSIS IN RAT BRAIN SLICES

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A low-molecular weight substance has recently been isolated from bovine brain and other tissues which potently displaces ^{3}H-clonidine from binding sites on brain membranes and modifies systemic blood pressure when injected into rat brain. This agent, termed clonidine-displacing substance (CDS), has been suggested to be an endogenous, clonidine-like ligand (Atlas & Burstein 1984). Here, we report preliminary investigations of one possible molecular mechanisms of action of CDS i.e. effects on the phosphoinositide (PI) hydrolytic cycle. CDS was isolated from bovine brain as described by Atlas and Burstein (1984). ^{3}H-clonidine binding to crude bovine synaptic membranes was performed as described by Rouot and Snyder (1979). The standard assay included 0.5 nM ^{3}H-clonidine and non-specific binding (<15% total) was defined by the presence of 100 μM noradrenaline. PI hydrolysis was assessed by measurement of ^{3}H-inositol phosphates (^{3}H-IP) accumulation in rat cerebro-cortical slices prelabelled with ^{3}H-myo inositol (^{3}H-MI) (Brown et al 1984). ^{3}H-MI uptake into rat cortical slices was measured using the method of Howerton and Rutledge (1988). CDS displaced ^{3}H-clonidine with simple mass action kinetics and the volume of extract which displaced 50% of specific binding in a 1 ml incubation volume was defined as one unit. The average yield was 1600±200 units/300 g brain (n=3). CDS (8.75 units/ml did not affect ^{3}H-prazosin or ^{3}H-quinuclidinyl benzilate binding.

In preliminary experiments CDS (8.75 units/ml) significantly inhibited ^{3}H-MI incorporation into the brain slice phospholipids (57±7% of control, n=3). The inhibitory effect of CDS on ^{3}H-MI incorporation into the phospholipids was accounted for by a reduction of ^{3}H-MI uptake into brain slices. Uptake was inhibited in a concentration-related manner with 50% inhibition in the presence of 2 units per ml of CDS (n=3). The method used was therefore modified to allow for this by washing out excess ^{3}H-MI before addition of CDS or other agents to the brain slices. Under these conditions (8.75 units/ml) CDS had no significant effect on ^{3}H-MI incorporation but increased ^{3}H-IP accumulation to 225±28% of control (n=3, $p<0.05$) after 45 mins incubation. In combination with noradrenaline (3×10^{-4} M) or the cholinergic agonist carbachol (10^{-3} M) the effects of CDS were no greater than additive (n=3).

The extract can therefore potentially modify the PI cycle by a positive effect on breakdown and by limiting the uptake of myo inositol which acts as a substrate for PI synthesis. It is not clear whether these effects are mediated by the same molecule and future studies will require additional purification of the CDS-containing extract.

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392P NON-α_2-ADRENOCEPTOR IDAZOXAN BINDING SITES ARE NOT INVOLVED IN THE PROCONVULSANT ACTION OF IDAZOXAN

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Idazoxan, a selective α2-adrenoreceptor antagonist, reduces seizure threshold to chemical convulsants in mice (Fletcher & Forster, 1988). Although this 'pro-convulsant' action is thought to be a consequence of increased noradrenaline release, it has recently been established that, in addition to α2-adrenoreceptors, idazoxan binds to so called 'non-adrenoceptor idazoxan binding sites' (NAIBS; Michel & Insel, 1989). In the current study we have further investigated the proconvulsant effects of idazoxan using an electroshock seizure threshold model, in mice. In addition, we examined the relative roles of α2-adrenoreceptors/NAIBS in this action by comparing idazoxan with two other potent α2-antagonists; efaroxan (Chapleo et al., 1984) and RX821002; (Langin et al., 1989) which do not bind to NAIBS (A.Hudson, personal communication).

Treatment (mg/kg ip)		CD50 (95% fiducial limits)
Saline		12.7(11.7 - 13.8)
Idazoxan	0.05	10.2(9.7 - 10.7)*
	0.5	11.2(10.2 - 12.2)*
	5.0	13.8(13.1 - 14.5)
RX821002	0.01	12.9(11.7 - 14.1)
	0.1	10.9(10.1 - 11.9)*
	1.0	10.9(10.1 - 11.9)*
Saline		11.7(11.1 - 12.4)
Efaroxan	0.05	10.3(9.8 - 10.8)*
	0.5	10.1(9.7 - 10.6)*
	5.0	10.2(9.7 - 10.7)*

* $p<0.05$ v corresponding vehicle

Electroshock seizure thresholds were assessed in groups of ten 30-40g male TO mice (Bantin & Kingman) using a constant current generator (0.5s, unipolar, via earclip electrodes) by the up and down method and probit analysis (Finney, 1971). Drugs were dissolved in 0.9% saline and administered ip 15 min before testing using a dose volume of 10ml/kg.

The current required to induce tonic hind-limb seizures in 50% of the sample (CD50) was significantly reduced by idazoxan (0.05, 0.5 mg/kg), efaroxan (0.05-5 mg/kg) and RX821002 (0.1, 1 mg/kg) as shown in the table. Since all three compounds lowered seizure threshold, the proconvulsant effects of idazoxan would appear to be due to antagonism of α2-adrenoreceptors and not to be mediated through NAIBS, a functional role for which has yet to be identified.

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393P THE EFFECT OF METHOHEXITONE ON THE RELEASE OF [^{3}H]-NORADRENALINE FROM RAT BRAIN SLICES

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The molecular basis of general anaesthesia has been studied for many years, but no definitive mechanism has been elucidated. In recent years much attention has focussed on inhibitory neurotransmitters. In particular, evidence suggests that a wide variety of chemically dissimilar general anaesthetics share the ability to potentiate the effects of GABA receptor stimulation (see Keane & Biziere, 1987). However, the potentiation by barbiturates of GABAergic activity may be related to anticonvulsant and anxiolytic effects rather than anaesthesia. Adrenergic mechanisms may also have a role in anaesthesia. A model has been proposed in which drug-induced alterations in the firing of locus coeruleus cells, or drug-induced changes in the post-synaptic effect of released noradrenaline, may be responsible for modulation of cortical arousal, wakefulness and the processing of sensory stimuli thus affecting the duration of barbiturate anaesthesia (Mason & Angel, 1983).

It has previously been shown that certain barbiturates inhibit release of GABA from rat cortical slices (but low concentrations can enhance release from thalamus) (Minchin, 1981; Kendall & Minchin, 1982). Preliminary experiments have been carried out to establish whether an effect could be detected on noradrenaline release from rat cerebral cortex. The method was based on that of Minchin & Pearson (1981). Release was stimulated by a pulse of 25 mM K^+. Results are expressed as the efflux rate for each 3 minute superfusion period. The increase in the efflux rate found during exposure to 25 mM K^+ was expressed as the increase over the mean basal efflux rate , and compared with drug-free controls run in parallel.

Methohexitone, at concentrations above 10^{-4}M, produced a dose-dependent decrease in [^{3}H]noradrenaline release. 10^{-4}, 3×10^{-4} and 10^{-3}M reduced release to 56.7 ± 10.5, 37.1 ± 9.2 and 8.9 ± 1.6% respectively (n = 6-9; $p<0.001$ for 10^{-3}M, Student's t test). A lower concentration (3×10^{-5}M) increased release to 204.9 ± 54.1% (n=3), but 10^{-5} decreased release to 28.7 ± 9.9% (n=3). Values are % control values, mean ± s.e. mean.

These preliminary results indicate that high concentrations of methohexitone decrease the K^+-stimulated release of noradrenaline in rat cortical slices. The effects at lower concentrations require further clarification. Studies will also be extended to a wider range of barbiturate and non-barbiturate anaesthetics to determine whether general anaesthetic potency correlates with inhibition of noradrenaline release.

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394P PRELIMINARY STUDIES OF THE ACTION OF 5-HT ON SINGLE LUMBAR MOTONEURONES IN THE SPINAL CORD OF THE NEONATE RAT

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Descending tryptaminergic pathways terminate in the vicinity of motoneurones (MNs) and of the afferents impinging on them (Holstege & Kuypers, 1987). 5-HT has complex actions on MNs (Elliott & Wallis, 1990). 5-HT produced concentration-dependent depolarizations with an increase in excitability and input resistance. At higher concentrations, 5-HT induced repetitive firing and, at lower concentrations, an increase in membrane noise. During application of 5-HT, spontaneous post-synaptic potentials and the response following dorsal root stimulation were markedly reduced. The pharmacology of these actions has been investigated using intracellular recordings from the hemisected spinal cord of 8-12 day old rats (Elliott & Wallis, 1990).

5-HT (10-300μM) produced depolarizations with an EC_{50} of 36 ± 9μM and a mean maximal response of 20 ± 2 mV (± s.e. mean, n=3-22, fitted curve to pooled data, Parker & Waud, 1971). In the presence of citalopram (0.1μM,>60 min), responses to 5-HT (0.3-30μM) were potentiated (EC_{50} 1.5 ± 0.4μM, maximum 17.3 ± 1 mV, n=3-8). Selective 5-HT receptor agonists were also tested (in the presence of citalopram unless otherwise stated). 5-CT, 0.1μM, had no effect on membrane potential but 10μM produced a depolarization of 5 mV. At both concentrations the response to dorsal root stimulation was markedly reduced. RU 24969 and 8-OH DPAT (both 10μM) had no effect on membrane potential. (±)α Me-5-HT (3-30μM) produced concentration-dependent depolarizations (EC_{50}≈10μM, and maximum 19 mV). (±)DOI, 10μM, in one experiment produced a 6 mV depolarization (no citalopram), whereas in others 10μM and 30μM (n=2) had no action. 2Me-5-HT at 30μM produced a mean depolarization of 5.7 ± 0.9mV (n=3) but paradoxically at 100μM no action (<1 mV, n=2). Ketanserin (1μM, 60 min) reduced 5-HT depolarizations by 63-90% (no citalopram); in the presence of citalopram 5-HT responses were reduced by 60% and 80% by 0.1 and 1μM ketanserin (60 min), respectively. Ritanserin (0.1μM, 60 min no citalopram) had no effect on 5-HT depolarization but did reduce the induced repetitive firing; at 1μM (60 min) the depolarization was reduced by 90%. ICI 169,369 (0.1μM, 20 min) had no effect on 5-HT responses.

In these preliminary experiments, the relatively high potency of (±)α Me-5-HT and the antagonistic action of ketanserin and ritanserin may suggest involvement of 5-HT_2 receptors in the depolarization. The actions of citalopram and possible indirect action of these agonists on MNs are being investigated further .

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395P THE EFFECT OF Ca^{2+} AND Mg^{2+} IONS ON THE BINDING OF [^{3}H]-Q ICS 205-930 TO THE 5-HT_3 RECOGNITION SITE IN RAT BRAIN WHOLE CORTEX

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Evidence for the existence of 5-HT_3 receptor binding sites in discrete regions of the rat brain has been demonstrated by high affinity binding of potent 5-HT_3 receptor antagonists such as [^{3}H]-GR 65630 (Kilpatrick *et al.*, 1987), and [^{3}H]-Q ICS 205-930 (Watling *et al.*, 1988). This study has investigated the differential effects Mg^{2+} and Ca^{2+} ions have on the ability of agonists and antagonists to displace [^{3}H]-Q ICS 205-930 from its recognition site in rat brain whole cortex. A modified method of [^{3}H]-Q ICS 205-930 (0.5-0.7 nM) binding to rat cortical membranes (Watling *et al*, 1988) was used. The selective 5-HT_3 receptor antagonist MDL 72222 (10 μM) was used to define specific binding (50-60% of total binding). Assays were carried out in Hepes buffer ± $CaCl_2$ (2.5 mM) and Mg SO_4 (1.2 mM). In the absence of Ca^{2+} and Mg^{2+} ions, both agonist and antagonist yielded shallow displacement curves, suggesting the presence of two sites/states. The addition of Ca^{2+} and Mg^{2+} ions to the incubation buffer caused the antagonist displacement curves to be shifted to the left with a subsequent increase in affinity. Conversely, the agonist displacement curves were shifted to the right with a resulting decrease in affinity. In both cases the slope of curves was increased with Hill coefficients (nH) approaching one.

	HEPES			HEPES + Ca^{2+} and Mg^{2+} ions		
	pK_i	nH		pK_i	nH	
ANTAGONISTS						
Zacopride	9.27 ± 0.25	0.56 ± 0.17	(n=3)	9.68 ± 0.12*	0.73 ± 0.29*	(n=3)
L-686,470+	9.25 ± 0.10	0.59 ± 0.13	(n=3)	9.71 ± 0.06**	0.75 ± 0.16**	(n=3)
AGONISTS						
5-HT	6.81 ± 0.09	0.70 ± 0.09	(n=4)	6.37 ± 0.11**	1.09 ± 0.17**	(n=4)
2-Methyl 5-HT	6.23 ± 0.09	0.78 ± 0.07	(n=3)	6.04 ± 0.06*	0.90 ± 0.07	(n=3)

Data are mean ± s.d. Significance of differences with respect to controls: * $p \leq 0.1$** $p \leq 0.01$ (Paired Student's t-test).
+exo 5-(1-Azabicyclo[2.2.1]heptan-3-yl)-3-(1-methyl-1H-indol-3-yl)-1,2,4-oxadiazole oxalate.

Recent evidence indicates that the 5-HT_3 receptor may be ion channel linked (Derkach *et al.*, 1989). These results suggest that agonists and antagonists bind to interacting sites within such a receptor complex, and that Ca^{2+} and Mg^{2+} ions cause the agonist binding site to revert to a lower affinity state and the antagonist binding site to a higher affinity conformation.

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396P PHARMACOLOGICAL COMPARISON OF THE RAT AND GUINEA-PIG CORTICAL HIGH AFFINITY 5-HT UPTAKE SYSTEM

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Unlike the rat high affinity 5-hydroxytryptamine (5-HT) uptake system, the guinea-pig system has only been subjected to limited pharmacological characterisation (Kilpatrick *et al*, 1986). The present studies therefore extend this pharmacological characterisation and compare it to that present in the rat.

Female Hooded-Lister rats (250-300g, Bradford bred) and male or female guinea pigs (500-900g, Dunkin Hartley, Bradford bred) were killed by cervical dislocation and the cerebral cortex was dissected and homogenised in 20 volumes of 0.32 M sucrose using a homogeniser with a teflon pestle (clearance 0.11-0.15 mm). The homogenate was centrifuged at 1000 g at 4°C for 10 min, the pellet discarded and the supernatant recentrifuged at 48000 g for 10 min. The pellet was gently resuspended in 0.27 M sucrose to form the crude synaptosomal preparation. High affinity [^{3}H]5-HT uptake was initiated by the addition of 250 μl of the crude synaptosomal preparation to pre-incubated (2 min, 37°C) test tubes containing 650 μl of competing compound (range of at least 7 concentrations) or vehicle (artificial CSF, mM; NaCl 126.6, $NaHCO_3$ 27.4, KCl 2.4, KH_2PO_4 0.49, $CaCl_2$ 1.2, $MgCl_2$ 0.83, Na_2HPO_4 0.49, glucose 7.1; also containing 10.0 μM pargyline; pre-gassed for 60 min with 95% O_2/5% CO_2) and 100 μl [^{3}H]5-HT (12.5-25.4 Ci $mmol^{-1}$, final concentration 10 nM). Uptake was allowed to proceed at 37°C for 6 min before termination by rapid filtration through Whatman GF/B filters followed by immediate washing for 12 sec with ice-cold artificial CSF. Protein estimation was performed by the method of Bradford (1976) using bovine albumin as the standard.

High affinity [^{3}H]5-HT uptake (defined by citalopram, 10.0 μM; approximately 80% of total [^{3}H]5-HT uptake) was 0.36 ± 0.1 and 0.27 ± 0.02 pmol min^{-1} mg^{-1} protein in the synaptosomes prepared from the rat and guinea pig cortex, respectively (mean ± S.E.M., n = 3). In both rat and guinea pig cortex inhibiting compounds reduced [^{3}H]5-HT uptake to a similar level as citalopram with a range of affinities (pIC_{50}, rat : guinea pig, mean ± S.E.M., n = 3); citalopram (7.83 ± 0.13 : 6.81 ± 0.09, fluoxetine (6.60 ± 0.10 : 6.64 ± 0.06), imipramine (6.43 ± 0.03 : 7.17 ± 0.03), mazindol (6.32 ± 0.10 : 6.01 ± 0.12), cocaine (6.20 ± 0.10 : 6.70 ± 0.00), amitriptyline (6.13 ± 0.07 : 7.00 ± 0.00), 8-OHDPAT (6.08 ± 0.04: 6.00 ± 0.00) GBR 12909 (5.84 ± 0.09 : 5.48 ± 0.04), desipramine (5.60 ± 0.06 : 6.03 ± 0.03), hemicholinium-3 (5.52 ± 1.00 : 4.93 ± 0.12), benztropine (4.84 ± 0.09 : 5.05 ± 0.05), nomifensine (4.81 ± 0.23 : 4.75 ± 0.34).

The present results suggest that the guinea pig high affinity 5-HT uptake system is similar in pharmacological specificity to that of the rat. However, there are some exceptions; most notably citalopram which is some 10 times weaker to inhibit 5-HT uptake in the guinea pig cortex compared to rat cortex.

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397P EFFECT OF A CHRONIC RITANSERIN REGIMEN ON AMINE AND METABOLITE LEVELS IN RAT FRONTAL CORTEX AND CEREBELLUM

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Chronic administration of the $5HT_2$ receptor antagonist, ritanserin (10 mg/kg/day) causes $5HT_2$ receptor down regulation (Leysen et al, 1986; Twist et al, 1990). On the basis of this finding, we examined the effects of the same drug regimen on amine and metabolite levels in frontal cortex and cerebellum.

Wistar rats (400g) received ritanserin or tartaric acid (50mM, pH = 5, vehicle) for 28 days. After a 3 day drug free period, animals were sacrificed, brains rapidly removed and frontal cortices and cerebella dissected on ice. Tissue homogenates, in 1 ml perchloric acid (0.1 M), were centrifuged (13,000 g x 10 min, 4°C), and the supernatant was analysed for 5HT, NA, DA, 5HIAA, DOPAC and HVA using HPLC-ED. Amine and metabolite levels, expressed as % change from control (ie, 100%) ± SEM, are shown in Table 1. and utilisation ratios (metabolite/amine) ± SEM for 5HT and DA are shown in Table 2.

TABLE 1.

	RITANSERIN	
	Cortex	Cerebellum
5HT	130.2 ± 4.3*	317.8 ± 80.9*[a]
NA	133.2 ± 6.7*	126.2 ± 17.3
DA	126.2 ± 12.6	368.0 ± 171.1
5HIAA	78.4 ± 6.3	117.4 ± 17.3
HVA	76.6 ± 6.5	122.2 ± 24.64
DOPAC	60.2 ± 9.65	122.2 ± 36.95

TABLE 2.

	CORTEX		CEREBELLUM	
	Control	Ritanserin	Control	Ritanserin
5HT	0.99 ± 0.11	0.58 ± 0.06 **	1.46 ± 0.01	0.90 ± 0.26 [a]
DA	0.41 ± 0.05	0.21 ± 0.02 **	2.84 ± 0.68	1.17 ± 0.26 *

(Data were analysed using unpaired t-tests, * $P<0.05$, ** $P<0.01$; n = 5 except [a] where n = 10)

Ritanserin significantly increased 5HT levels in the cerebellum; but had no significant effect on any of the other amines or metabolites. There was a large increase in DA but due to the high SEM this was not significant. In cortex, amines were increased and metabolites decreased and, in the case of 5HT and NA, these changes were significant. The increase in 5HT might be causally related to $5HT_2$ receptor down regulation, however, it is possible that the increase in 5HT does not occur in the synapse. This is suggested by the utilisation ratio for 5HT which was significantly decreased in cortex ie, ritanserin appears to increase stored amine. This maybe due to a drug induced decrease in neuronal firing but this is difficult to explain in terms of the ability of ritanserin as a $5HT_2$ receptor antagonist and suggests that the drug has other, as yet unknown, effects.

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398P THE PROTECTIVE EFFECT OF BRL 43694 AGAINST CYTOSTATIC TREATMENT IN 10-13 WEEK OLD FERRETS

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BRL 43694 has been shown to protect against the characteristically severe 'nausea' and vomiting responses to X-irradiation or cytotoxic drugs in ferrets (Bermudez et al, 1988) and man (Carmichael et al, 1988). However, it was not known whether very young animals or children would receive similar protection with BRL 43694. This work was conducted to examine responses in the weanling ferret and compare them to those in adult ferrets.

Intravenously-cannulated 10-13 week old male ferrets (fitch or albino) received a single dose of BRL 43694 (BRL; 0.5 mg/kg iv) or vehicle (V) 15 min before (i) 10.4 min exposure to X-irradiation, or (ii) cyclophosphamide + doxorubicin (80 + 6 mg/kg iv respectively). The time to the first vomit (latency period) was recorded, and the number of emetic episodes was monitored for 120 min (i) or 240 min (ii). Results were compared with data from adult ferrets.

Emetogenic stimulus	Group	Latency Period (min)	Emetic Episodes	No of animals Vomiting
(i) X-irradiation	Weanlings + V.	24.0± 2.4	19.0±6.7	4/4
(10.4 min)	Weanlings + BRL	**92.0±16.7	*1.3±0.8	2/4
	Adults + V.	22.8± 0.8	14.3±1.6	6/6
(ii) cyclophosphamide	Weanlings + V.	103.0±15.9	4.0±0.9	4/4
doxorubicin	Weanlings + BRL	**220.3±19.8	2.3±2.3	1/4
(80:6 mg/kg iv)	Adults + V.	55.7±16.1	20.6±3.9Δ	12/12

Means ± SEM, Student's 't' test. *$p<0.05$; **$p<0.01$; vehicle v BRL 43694.
Δ$p<0.05$; adults vs weanlings. If no vomiting occurred, the latency was taken as 120 (i) or 240 (ii) min.

BRL 43694 was well tolerated by weanling ferrets and gave good protection from a strongly emetic dose of X-irradiation. BRL 43694 also abolished vomiting induced by cyclophosphamide and doxorubicin in 3 out of 4 weanling ferrets and reduced it in the remaining animal. Although the response of the weanlings to X-irradiation was the same as that of the adult, it was interesting to find significantly reduced vomiting in the weanlings given cytotoxic drugs. In a further experiment, cisplatin (12.5 mg/kg iv; data not shown) failed to induce vomiting in 2 other weanlings, suggesting that the weanlings may have reduced sensitivity to cytotoxic drugs. It is concluded that BRL 43694 is able to abolish or greatly reduce vomiting induced by either X-irradiation or cytotoxic drugs in both adult and weanling ferrets. These data provide encouragement to conduct paediatric clinical studies with BRL 43694 against cytotoxic drug-induced emesis.

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399P QUALITATIVE AND QUANTITATIVE ESTIMATION OF DOPAMINE AND 5-HT IN MIXTURES ASSESSED BY FAST CYCLIC VOLTAMMETRY (FCV) AND NUMERIC ANALYSIS

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Fast cyclic voltammetry (FCV) is an electrochemical detection method which has been used to measure the overflow of electrically stimulated dopamine in vivo and in brain slices (Millar et al 1985, Palij et al 1990). The method depends on being able to monitor the oxidation peak potential for dopamine. To date the method has been used in brain regions where dopamine is the most abundant transmitter. To establish whether it would be feasible to monitor concurrently both dopamine and 5-HT released at sites where they are present in equal abundance, a series of in vitro experiments were conducted using standard solutions and mixtures of these compounds and subjecting the data to numeric analysis (Mas et al, 1989; Derbtein & Helmer, 1988).

Solutions of dopamine or 5-HT (10^{-7} or 10^{-6} M) in artificial cerebrospinal fluid (Palij et al 1990) or mixtures of known composition were studied by FCV. The electrochemical signals were digitised with 12 bit resolution at 40 KHz using a CED 1401 interface running SIGAVG. For each separate compound, and for each mixture, 10 background and 10 sample signals were recorded at each concentration. Following subtraction of background (charging) current, the Faradaic current array for each scan was edited so that it contained only the oxidation peak. The rising phases of the oxidation currents for both compounds had the properties of a gausian curve. The peak oxidation potentials and the standard deviations of the waveform signals of dopamine and 5-HT were significantly different. When standard mixtures were analyzed numerically, using expressions which describe a gausian with two distinct components, then the presence of both compounds could be demonstrated qualitatively and the individual contributions of each compound quantified. These experiments demonstrate that it may be possible to estimate the nature and quantities of monoamines contributing to an apparently single peak in vivo or in brain slices. The data acquisition, handling and analytical routines are realised on a PC compatible machine.

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400P *IN VITRO* ANALYSIS OF STRIATAL ELECTROACTIVE COMPOUNDS WITH DIFFERENTIAL PULSE VOLTAMMETRY AND CARBON FIBRE MICRO ELECTRODES

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Differential pulse voltammetry (DPV) with electrically pretreated carbon fibre micro electrodes (CFE) has been successfully employed for the *in vivo* analysis of neurotransmitter release and metabolism (Crespi *et al.*, 1984 & 1988). We have now explored the feasibility of applying this methodology to *in vitro* preparations; using striatal slices prepared from adult rat brain. The present study investigates the use of such slices for the *in vitro* measurement of dopamine (DA) metabolism.

The striatal slices (500 μm) were prepared using a manually operated tissue slicer previously described (Bennett *et al.*, 1983). The three electrodes needed for DPV (auxiliary, reference and 12 μm diameter CFE) were stereotaxically inserted in the striatal slice which was maintained in a slice chamber at 34°C, pH 7.4 in Krebs medium under 95% oxygen, 5% carbon dioxide. DPV measurements (scans) were made every 5 min and two oxidation peaks detected simultaneously at each scan: Peak 1 at - 70 mV and Peak 2 at + 30 mV. While both signals were stable up to 3 hours following addition of saline (NaCl 0.9%) to the perfusate, only Peak 2 was largely decreased (90%) by pargyline (50 μM), slightly decreased (20%) by amphetamine (50 μM) and not altered by the addition of K+ (KCl 25-100 mM). These data indicate that Peak 2 mainly results from the oxidation of extracellular DOPAC (Gonon *et al.*, 1981). While further experiments need to be performed to support this finding, these results indicate the capability of DPV with 12 μm CFE to analyse electroactive DA metabolites in cerebral slices but not that of K+ stimulated release of DA, further supporting the view that the DA metabolite DOPAC cannot be used as an index of DA release. The use of the 30 μM CFE coated with Nafion (Crespi *et al.*, 1988) together with the electrically pretreated CFE should allow the detection of the neurotransmitter itself and its metabolite DOPAC.

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401P EXTRACELLULAR DOPAMINE CONCENTRATION IN RAT STRIATUM FOLLOWING TETRAHYDROAMINOACRIDINE ADMINISTRATION

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Tetrahydroaminoacridine (THA) is a cholinesterase inhibitor which may be useful for the treatment of senile dementia. In addition to its cholinergic actions, THA stimulates the release of dopamine from rat brain slices (Robinson et al., 1989). We have used intracerebral dialysis to study the release of dopamine from rat striatum in vivo.

Rats (Male Lister Hooded, 200g-300g) were anaesthetised with pentobarbital and a concentric dialysis probe (4μm x 200μm) implanted stereotaxically into the striatum 24h before the start of the experiment (Adell et al., 1989). On the day of experiment rats were anaesthetised with chloral hydrate, artificial c.s.f. pumped through the dialysis probe at 1μl min^{-1}, and samples collected before and after THA (5mg kg^{-1} i.p.) administration. Dopamine concentrations were determined by h.p.l.c. (Robinson et al., 1989), no correction was made for recovery through the probe.

THA (5mg kg^{-1} i.p.) produced marked tremor which was apparent for at least 1h following administration. Striatal extracellular dopamine concentrations were not altered by THA administration (Table 1).

Table 1. Extracellular dopamine concentration in rat striatum.

	Dopamine (ng sample)		
	Basal	K^+ stimulated	Amphetamine 5mg kg^{-1}
Control	0.50 ± 0.06	2.32 ± 0.60	5.82 ± 1.10
THA	0.45 ± 0.06	2.61 ± 0.91	4.61 ± 0.83

Values are mean ± s.e.m., n=4-10.

The release of dopamine induced by a 30 minute pulse of artificial c.s.f. containing 100mM KCl was not altered by THA administration (Table 1).

Amphetamine (5mg kg^{-1} i.p.), administered at the end of the experiment, produced a substantial release of dopamine in all animals. The present data demonstrate that the dopamine-releasing action of THA observed in vitro is not apparent in vivo at doses of THA producing marked cholinergic behavioural effects.

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402P REVERSIBLE DOPAMINE DEPLETION FOLLOWING UNILATERAL INFUSION OF MPTP IN THE COMMON MARMOSET

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Following peripheral administration or unilateral infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into the substantia nigra of the common marmoset, the induced parkinsonism behavioural syndrome is reversible (Jenner *et al*, 1986, Gerrard, unpublished data). The present study investigates whether the neurochemical changes in the dopaminergic system can also be reversed.

Male or female common marmosets (Callithrix jacchus) weighing 350-400g were subject to unilateral infusion of MPTP or vehicle (0.9% w/v NaCl)) into the substantia nigra (0.5μl/h for 14 days; 20μg/24h). The animals were killed by decapitation at various time periods after the MPTP/vehicle treatments. Levels of neurochemicals in dissected brain regions were assayed by HPLC with electrochemical detection by the method of Barnes *et al*, 1988.

Table 1. The effects of unilateral infusion of MPTP into the substantia nigra on the striatal levels of dopamine at various time periods after withdrawal of MPTP treatment.

Brain region	Side	Vehicle	Time 1D	3M	5M	8M	12M	18M
Caudate	Ipsilateral	462.0±257	156.8±140	287.5±127	94.2± 87*	249.4	472.9	344.9
	Contralateral	347.7±131	638.8± 46	728.0± 98	840.6±121	986.7	424.3	463.4
Putamen	Ipsilateral	336.3± 49	30.5± 27*	233.3±112	96.4± 94	138.4	30.3	297.5
	Contralateral	303.0± 42	364.9± 76	561.8± 89	459.5± 88	811.5	139.3	454.0

Data are expressed in ng/mg protein, mean ± S.E.M. (n = 3-5) or mean (n = 2), D = day, M = month. Significant difference compared to the corresponding vehicle values, *P<0.05 (Mann-Whitney U test).

The intranigral administration of MPTP initially caused large reductions in dopamine levels (60-90%) from day 1 to 5 months after MPTP treatment in the ipsilateral side of the caudate and putamen whilst it increased levels in the contralateral side. Between 12 and 18 months after treatment DA levels returned towards the vehicle levels. The results suggest that dopaminergic depletions following the unilateral central infusion of MPTP in the marmoset are reversible.

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403P PRESYNAPTIC DOPAMINE UPTAKE SITES LABELLED BY [^{3}H]-GBR 12935: EFFECTS OF UNILATERAL 6-HYDROXYTRYPTAMINE LESIONS OF RAT SUBSTANTIA NIGRA

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Janowsky et al. (1986) proposed that [3H]GBR 12935 selectively labels the dopamine (DA) transport complex in the brain. Evidence for binding to presynaptic sites was provided by intraventricular lesioning with 6-hydroxydopamine (6OH-DA). However, the very high (200μg x 2) dose used, the route of administration and the incomplete nature of the lesion cast doubt on this conclusion. We have now determined the effects of unilateral injection of 6OH-DA into the substantia nigra of rats on methamphetamine-induced circling responses and also the relative reductions in [3H]GBR 12935 binding and DA content (each unlesioned striatum acting as a control).

Male CD rats (275-300g) were given unilateral substantia nigra lesions using various doses of 6OH-DA (2-8μg), while controls received saline-ascorbate (4μl). Animals were left to recover for 3 weeks. Circling responses to methamphetamine (2 mg/kg ip) were determined on ≥3 separate occasions 2-3 weeks before removing and dividing individual, lesioned and unlesioned striata. [3H]GBR 12935 (0.1-10nM) binding, defined by 1μM GBR 12909, was measured in posterior striatal membranes. DA concentrations were determined in anterior striata by HPLC-ECD.

Table 1 Effects of unilateral nigral 6OH-DA lesions on circling, [3H]GBR 12935 binding and DA levels in rat striatum

6OH-DA dose (μg)	Circling (turns/min)	%decrease in [3H]GBR 12935 binding	%decrease in DA levels
0	0.3 ± 0.25	6 ± 13	4 ± 9
2	1.7 ± 1.7	49 ± 4**	61 ± 10**
4	8.3 ± 2.1#	61 ± 12*	90 ± 5**
8	8.3 ± 0.6#	95 ± 5**	97 ± 2**

Values are mean ± s.e. mean (n=3-4). # $p<0.05$ vs ascorbate control. * $p<0.05$, ** $p<0.01$ vs unlesioned striatum.

6OH-DA injection produced dose-dependent increases in methamphetamine circling showing a progressive functional imbalance between lesioned and unlesioned striata. A ≥70% reduction in DA levels was required for the appearance of this response. Mean [3H]GBR 12935 binding in the unlesioned striata was 27.3 ± 6.6 fmol/mg protein (n=15). 6OH-DA progressively diminished binding on the lesioned side, accompanied by parallel losses of DA. However, 6OH-DA lesioning also revealed a second specific binding site for [3H]GBR 12935 with moderate affinity and very high capacity; this restricted the concentration range for saturation binding analysis. Hence, although the data support the hypothesis that [3H]GBR 12935 labels the DA transport complex, they also show that in 6OH-DA-lesioned striata this ligand specifically binds to a second site which is not on presynaptic DA nerve terminals.

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404P A COMPARISON OF THE MONOAMINE REUPTAKE INHIBITION AND α_1- AND MUSCARINIC RECEPTOR BINDING PROPERTIES OF DOTHIEPIN, ITS METABOLITES AND OTHER ANTIDEPRESSANTS

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Dothiepin is a tricyclic antidepressant which inhibits noradrenaline (NA) and 5-hydroxytryptamine (5-HT) reuptake. In this study, we have determined the relative contributions of dothiepin and its major metabolites to uptake inhibition and compared them with doxepin and amitriptyline which are both chemically similar to dothiepin. In addition, the potential side-effect profile resulting from inhibition of alpha$_1$- and muscarinic receptors has been assessed.

Inhibition of synaptosomal uptake of 10nM [3H]NA and 2nM [3H]5-HT (rat cortex; 2.5 and 1.25 mg/tube respectively) and 2.5nM [3H]dopamine (DA) (rat striatum; 0.625 mg/tube) was measured as described by Cheetham et al., (1990). Affinities for alpha$_1$- and muscarinic receptors were determined by displacement of [3H]prazosin (0.5nM) and [3H]N-methylscopolamine (0.15nM) binding to rat cortical membranes defined by phentolamine (5μM) and atropine (1μM).

Table 1: The effects of dothiepin, its metabolites and other antidepressants on uptake and receptor binding

Antidepressant	[3H]NA	[3H]5-HT	[3H]DA	alpha$_1$	Muscarinic
Dothiepin	70 ± 3	78 ± 5	5185 ± 226	419 ± 27	26 ± 1
Northiaden	25 ± 1	192 ± 13	2539 ± 232	950 ± 200	110 ± 8
Dothiepin sulphoxide	4912 ± 640	5402 ± 76	77,386 ± 4123	9660 ± 1683	2512 ± 427
Northiaden sulphoxide	1948 ± 176	534 ± 33	58,727 ± 7318	12,151 ± 1908	5478 ± 101
Doxepin	18 ± 2	201 ± 17	8282 ± 377	27 ± 4	40 ± 2
Amitriptyline	28 ± 1	49 ± 2	3780 ± 234	44 ± 16	10 ± 1

Values are mean K_i's ± s.e. mean (nM) for 3 - 6 determinations.

Dothiepin inhibited NA and 5-HT uptake equally, whereas northiaden preferentially blocked NA uptake. However, their sulphoxides were both ineffective. As human plasma levels of northiaden are comparable with dothiepin (Yu et al., 1986), this metabolite probably contributes significantly to the therapeutic effects of dothiepin. Dothiepin and its metabolites had markedly lower affinity for alpha$_1$-adrenoceptors than the other antidepressants. Dothiepin, northiaden and doxepin had respectively 2.6, 11 and 4 times less affinity for muscarinic receptors than amitriptyline. Furthermore, the sulphoxides had no significant affinity for muscarinic sites. These data, therefore, suggest potentially less alpha$_1$-adrenergic and muscarinic side-effects for dothiepin and northiaden than for doxepin and amitriptyline.

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405P ONLY ANTIDEPRESSANTS WITH MARKED EFFECTS ON CENTRAL DOPAMINE FUNCTION DECREASE RAT STRIATAL D_2 RECEPTOR NUMBER

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Klimek & Nielsen (1987) claimed that repeated antidepressant administration decreased dopamine D1, but did not affect D2 receptors. In contrast, Martin et al (1988) found no change in D1 receptors after similar treatments. In this study, we report the effects of antidepressant treatments on D2 receptors in the rat striatum (CPU).

Male CD rats (100-125g) received drugs (see Table 1) or saline i.p. once daily for 1 or 14 days. Halothane anaesthetised rats were given either one electroconvulsive shock (ECS; 200 v, 2 s), or 5 ECS over 10 days. CPU were removed 24 h after the last treatment. D2 binding parameters were determined using [3H]raclopride (six concentrations 0.3-10 nM) with specific binding defined by 1 μM sulpiride (Martin et al, 1990). Data were analysed by Students t-test.

Single treatments had no effects on D2 receptor binding parameters (data not shown). Repeated administration of nomifensine and tranylcypromine decreased D2 receptors but other antidepressants and ECS had no effect (Table 1). Haloperidol increased D2 receptor number confirming that changes in [3H]raclopride binding reflect changes in D2 receptors. Kd values were unaltered by all treatments.

Table 1: The effect of repeated antidepressant treatments and haloperidol on D2 receptor number in the rat CPU

Treatment	Bmax	Treatment	Bmax	Treatment	Bmax
Pooled Control (n=48)	475±8	Zimeldine (10)	461±19	Haloperidol (1)	554±24***
Sibutramine HCl (3)	454±12	Nomifensine (5)	417±11**		
Amitriptyline(10)	466±34	Mianserin (5)	481±17	Halothane (x 5) Control	572±12
Desipramine (10)	491±20	Tranylcypromine (10)	425±15**	ECS (x 5)	587±18

Dose (mg/kg) in parentheses. Mean±s.e.mean (fmol/mg protein, n=8-12). **P<0.01,***P<0.001 vs. own control.

Our findings generally support the view of Klimek & Nielsen (1987) that most antidepressants do not alter D2 receptors. However, drugs which enhance dopamine function do down-regulate these receptors. The lack of effect of ECS on D2 binding agrees with functional studies reported by Newman & Lerer (1989).

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406P MIDBRAIN SECTION IN THE FERRET MODIFIES DRUG-INDUCED EMESIS

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Early investigations into the localisation of a vomiting centre utilised decerebrate preparations since anaesthesia was known to severely depress the vomiting reflex. The technique effectively abolishes all input from forebrain structures to the brainstem, and agents that induce emesis by an action on forebrain structures are rendered ineffective (Brand *et al.*, 1953). Here we further investigate the influence of the forebrain to drug-induced emesis in the ferret using a midbrain section.

Female albino or fitch ferrets (0.9-1.3kg) were sectioned under halothane anaesthesia (N_2O/O_2 carrier) using a modified spatula secured in a Kopf tool carrier (co-ordinates used were posterior 4.4 to 4.6 with the incisor bar raised 5mm above zero). Animals were left to freely ventilate for 2 hrs before any further experimental procedure. Normal and midbrain-sectioned animals were administered apomorphine (0.25mg/kg s.c.), copper sulphate ($CuSO_4.5H_2O$ 100mg/kg intra-gastrically) and cisplatin (10mg/kg i.v.). The control non-sectioned animals were chronically cannulated for the administration of cisplatin.

Table 1. The emetic response of apomorphine, copper sulphate and cisplatin in control (C) and midbrain-sectioned (S) ferrets during a 30, 30 and 240min period respectively post drug treatment.

Treatment	State	n	Onset of emesis (min)	No. episodes	No. retches	No. vomits
Apomorphine	C	6	1.9±0.3	6.8±1.1	29.7±5.5	4.3±0.6
0.25mg/kg	S	4	15.9±8.2	2.0±1.4	11.2±10.6	0.5±0.5*
$CuSO_4.5H_2O$	C	4	5.4±0.1	12.3±1.9	61.8±13.3	8.8±1.4
100mg/kg	S	3	2.1±0.9	9.7±0.9	64.7±4.9	4.3±0.7
Cisplatin	C	6	76.6±4.7	12.2±2.0	72.8±15.0	5.5±1.2
10mg/kg	S	6	110.7±10.1*	3.3±1.0**	17.7±4.1*	0.0±0.0*

Each value is the mean ± S.E.M. Significant delays in the onset of emesis or changes in the number of episodes, retches/vomits of midbrain-sectioned as compared to control animals is indicated as *<0.05; **<0.01 (Mann-Whitney U test).

Copper sulphate-induced emesis was not significantly antagonised by the midbrain section, although there was a clear trend for the severity of apomorphine-induced emesis to be reduced, with the lesion causing a significant reduction in the number of vomits. All the parameters of cisplatin-induced emesis were significantly antagonised in the lesioned ferret.

In conclusion, forebrain participation would appear more significant for apomorphine and particularly cisplatin-induced emesis than for copper sulphate. The degree to which these results may reflect a lesion-induced disruption of blood flow to the hind brain is being assessed.

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407P INVOLVEMENT OF CENTRAL DOPAMINE AND CCK_A RECEPTORS IN THE APOMORPHINE-INDUCED HYPOTENSIVE RESPONSE IN THE ANAESTHETISED RAT

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Many actions of apomorphine are due to stimulation of dopaminergic receptors but there is conflicting reports about the role of central dopamine receptors in apomorphine-induced hypotension (Finch & Haeusler, 1973). A significant number of midbrain dopamine cells also contain the peptide cholecystokinin (CCK) (Hökfelt et al, 1980). The development of selective CCK antagonists; L-364,718 for CCK-A and L-365,260 for CCK-B (Lotti & Chang, 1989) has made it possible to investigate the involvement of CCK in apomorphine-induced effects in the anaesthetised rat.

Male Wistar rats were anaesthetised with urethane, and cannulated for measurement of blood pressure and i.v. administration of all drugs. Intravenous injections of apomorphine (10-1000nmol kg^{-1}) or sulphated CCK-8 (2.5-10 nmol kg^{-1}) produced short lasting dose dependent falls in blood pressure. Apomorphine-induced depressor actions could be antagonised by the dopamine antagonist, haloperidol (0.1-10 µmol kg^{-1}) but not by domperidone (2-10µmol kg^{-1})(n=10). Pretreatment with L-365,260 (1-3µmol kg^{-1}) reduced hypotensive responses to CCK-8 but had no effect on apomorphine or dopamine blood pressure responses whilst L-364,718(1-2µmol kg^{-1}) pretreatment antagonised both CCK-8 and apomorphine-induced responses in all animals (n=12) (Table 1). This antagonism was surmountable with higher doses of apomorphine.

Table 1 shows median depressor responses (mm Hg) in anesthetised rat (n=12) after i.v. injection of apomorphine or sulphated CCK-8 before, and after L-365,260 or L-364,718, also administered i.v..

Depressor agent (nmol kg^{-1})	Control	Responses (range) mm Hg + L-364,718(2µmol kg^{-1})	+ L-365,260(3µmol kg^{-1})
Sulphated CCK-8 (2.5)	22 (20-30)	3 (0-8)	3.5 (0-10)
Apomorphine HCl (30)	7.5 (5-10)	0	10 (8-20)
Apomorphine HCl (100)	20 (10-25)	5 (0-10)	20 (17-35)
Apomorphine HCl (300)	33 (20-40)	18 (15-25)	35 (30-40)

These results suggest that both CCK-A and central dopamine receptors mediate apomorphine-induced depressor responses in the anaesthetised rat.

We wish to thank Merck, Sharpe & Dohme Research Laboratories for the gifts of L-365,260 and L-364,718.

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408P A NOVEL WAY OF PRESENTING THE STATISTICAL ANALYSIS OF LOCOMOTOR ACTIVITY

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Generally, analysis of locomotor activity (LMA) has been undertaken in the original units. It has been suggested that by taking logarithms the validity of the psychological interpretation may be compromised (Robbins, 1977). Presentation of treatment means in terms of ratios and their confidence intervals overcomes the difficulties of interpreting differences on a logarithmic scale. We have re-assessed the statistical methodology used in the analysis of LMA to allow the analysis of data from unbalanced experimental designs and produced novel ways in presenting the subsequent results. The methods are illustrated using data from an experiment to investigate the effects of SK&F 81297.

The LMA was measured in a bank of 4 activity cages. Each rat received a single treatment and data was automatically recorded for 60 min. The experiment consisted of 52 animals in 13 batches of 4. Total LMA over the 60 min period was used as the primary parameter of interest. Individual LMA data was plotted to assess the variability. Variation increased with the magnitude of response, resulting in loss of power and the invalidation of assumptions for statistical procedures. The logarithmic transformation was employed to overcome these problems.

Analysis of variance (ANOVA) was performed taking into account the cage and batch in which the animal was studied and the treatment they received. Treatment means were first adjusted to account for the imbalanced design and then back-transformed from the logarithmic scale to give geometric means in the original units. Comparisons between the treatment and vehicle means were performed by calculating differences and 95% confidence intervals, again on the logged data. When back-transformed to the original units differences in mean values were converted back to ratios of means (Figure 1).

Logarithmic transformation stabilized the variability and increased the power in identifying treatment differences. The transformed data also meets statistical assumptions and results can easily be interpreted by the examination of ratios and their confidence intervals.

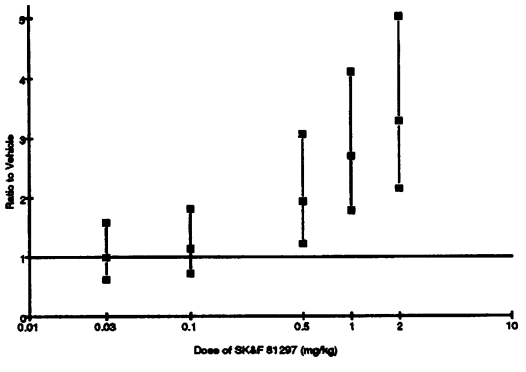

Figure 1 Ratios of Treatment Group Means to the Vehicle with 95% Confidence Intervals.

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409P COMPARISON OF THE EFFECTS OF SELECTIVE D_1 AGONISTS ON DOPAMINE-MEDIATED BEHAVIOUR

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The majority of agonist studies investigating the functional role of the D_1 receptor have used SKF 38393, a partial D_1 agonist. This has no effect on the spontaneous locomotor activity (LMA) of intact animals (Koller and Herbster, 1988). The extent to which this observation is characteristic of D_1 agonists can now be investigated following the development of full agonists selective for the D_1 receptor Arnt et al 1988). In the present study we have compared the effects of SKF 38393 with those of the selective full D_1 agonist SKF 81297.

Male Lister-Hooded rats (250-300g) were habituated to activity cages for 60 mins prior to test. Drugs were administered and LMA was recorded for a further 60 min. During this time, stereotyped behaviour exhibited by each animal was assessed for 1 min in each 5 min interval. Each animal received a single dose of either vehicle, SKF 38393 (10 or 30mgkg^{-1}) or SKF 81297 (0.1-2.0mgkg^{-1}). A separate group of animals received unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway and were allowed three weeks to recover. Each animal then received a balanced dose range of either SKF 81297 (0-2.0mgkg^{-1}) or SKF 38393 (0-16.0 mgkg^{-1}). The direction and number of rotations were recorded for 3 min, every 15 min for 2 hours post dosing. All drugs were administered s.c.

SKF 38393 had no effect on LMA [log.activity, $F(2,16)=1.66$ n.s.]. By contrast, SKF 81297 tested caused a dose-related increase in LMA [log.activity, $F(2,17)=5.06$ $p<0.05$](Table 1), which was reversed by SCH 23390 (0.05mgkg^{-1}). SKF 81297 caused an increase in the incidence of grooming but the effect was not as great as that seen with SKF 38393. No other stereotyped behaviour was observed. Both compounds produced contralateral rotation in the unilaterally lesioned animals with the minimum effective doses being 1.0mgkg^{-1} of SKF 38393 and 0.0625mgkg^{-1} of SKF 81297.

Table 1

mg/kg	veh	10.0	30.0		veh	0.1	1.0	2.0
SK&F 38393	6596±1271	8525±1103	9254±878	SK&F 81297	8377±1855	11244±1009	18918±3488*	19874±5079*

Results expressed as mean±s.e.m. of total light paths broken during 60min test. * $p<0.05$ compared with vehicle. Data analysed as described in Bond et al, this meeting.

These results show that SKF 81297 stimulates LMA in intact rats whereas SKF 38393 does not. Both compounds induce grooming behaviour and are effective direct dopamine receptor agonists as shown by the contralateral rotation of the unilaterally lesioned animals. The dissociation of behavioural effects seen with the partial agonist SKF 38393 and the full agonist SKF 81297 suggests that some re-appraisal of the role of the D_1 dopamine receptor subtype in the neostriatum may be necessary.

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410P EFFECTS OF THE 5-HT_2 RECEPTOR LIGANDS, DOI AND RITANSERIN, ON 5-HT_{1A} RECEPTOR-MEDIATED BEHAVIOUR IN THE RAT

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Recent behavioural studies suggest that central 5-HT_2 and 5-HT_{1A} receptors may be functionally linked. Arnt and Hyttel (1989) have reported that the 5-HT_2 agonist DOI (Glennon et al., 1986) facilitates the forepaw treading response to 8-OHDPAT in rats. However, Backus et al., (1989) have described enhanced behavioural effects of 8-OHDPAT (and other 5-HT_{1A} agonists) induced by ritanserin, a 5-HT_2 antagonist. In order to investigate this apparent inconsistency, we have examined the effects of DOI on the potencies of 8-OHDPAT, buspirone and gepirone to induce a behavioural syndrome in rats. We have also examined the effect of ritanserin both on the behavioural response to 8-OHDPAT alone, and on the enhanced response induced by DOI.

Drug or vehicle were administered subcutaneously to male Sprague-Dawley rats (350-400g) 30 min. before the intravenous administration of 5-HT_{1A} agonist. The behavioural syndrome (forepaw treading and flat posture) was scored quantally (unequivocal response or non-response) and the potencies of 5-HT_{1A} agonists were determined by the up/down method (Kimball et al., 1957) as described previously (Fletcher and Forster, 1989).

Table 1

Pretreatment (mg/kg s.c.)	Effects of DOI on the in vivo potencies of 5-HT_{1A} agonists: ED_{50} values (with 95% confidence limits; n = 10) (mg/kg i.v.)		
	8-OHDPAT	Buspirone	Gepirone
Vehicle	0.063 (0.054 - 0.074)	0.36 (0.26 - 0.48)	0.67 (0.58 - 0.77)
DOI (0.1)	0.041 (0.035 - 0.048)		
DOI (0.5)	0.015 (0.0093 - 0.026)		
DOI (2.5)	0.0025 (0.002 - 0.0031)	0.033 (0.022-0.050)	0.031 (0.020 - 0.049)

DOI markedly increased the potencies of 8-OHDPAT, buspirone and gepirone to induce a syndrome (Table 1). Ritanserin (0.1-3.0 mg/kg s.c.) had no significant effect on the potency of 8-OHDPAT. Ritanserin (0.1-1.0 mg/kg i.p.) also had no significant effect on the enhanced potency of 8-OHDPAT induced by DOI (2.5 mg/kg s.c.).

These data suggest that the facilitatory action of DOI on 5-HT_{1A} agonist-induced behaviour is either not mediated by 5-HT_2 receptors, or is mediated by a ritanserin-insensitive sub-type of the 5-HT_2 receptor.

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411P EFFECTS OF CAESIUM ON 5-HT MEDIATED BEHAVIOUR IN RAT AND MOUSE

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Caesium belongs, with sodium, potassium, lithium and rubidium, to the biologically-significant Periodic Group Ia alkali earth elements. We have shown that the effects of rubidium on 5HT-mediated behaviour in rats and mice are quite different to those of lithium (Wang & Grahame-Smith, 1990). Jenner & Judd et al. (1975) reported that treatment of rats with caesium chloride (CsCl) in the diet for 14 days followed by monoamine oxidase inhibition resulted in the 5HT behavioural syndrome. We have investigated further the effects of caesium on 5HT mediated behaviour in the rat and mouse. Sprague-Dawley derived rats (160-250 g) and C57/black mice (20-30 g) were given either CsCl (3 mmol/kg, s.c.) or saline (as control) twice daily for 3 days. The rats were observed and rated for the components of the behavioural syndrome (Deakin and Green, 1978). Locomotor activity was monitored on Opto-Varimex meters. The administration of tranylcypromine [TCP] (15 mg/kg, i.p.) to rats pretreated with CsCl (3 mmol/kg, s.c., twice daily for 3 days) produced the 5-HT behavioural syndrome. Identical treatment with CsCl also enhanced the syndrome induced by p-chloroamphetamine (3 mg/kg, i.p) or by TCP (15 mg/kg, i.p.) plus L-tryptophan (50 mg/kg, i.p.). The 5-HT synthesis inhibitor, p-chlorophenylalanine (300 mg/kg, i.p., daily on 2 consecutive days), and the 5-HT antagonists, (-)-propranolol (20 mg/kg, i.p.), pindolol (4 mg/kg, i.p.) and ritanserin (0.4 mg/kg, s.c.), all prevented the behavioural syndrome induced by CsCl and TCP in rats. Pretreatment of rats with CsCl potentiated the 5-HT syndrome elicited by the 5HT agonists, 8OHDPAT (0.5 mg/kg, s.c.), 5MeODMT (2 mg/kg, s.c.) and quipazine (25 mg/kg, i.p.) in rats. The head-twitch response of mice to carbidopa (25 mg/kg, i.p.) plus 5HTP (100 mg/kg, i.p.), or 5MeODMT (5 mg/kg, s.c.) or DOI (2 mg/kg, s.c.) were also potentiated by pretreatment of CsCl (3 mmol/kg, s.c., twice daily for 3 days). In conclusion, caesium affects 5HT-mediated behaviour in rats and mice in a different way from both lithium (Grahame-Smith, 1988) and rubidium (Wang & Grahame-Smith, 1990). The enhancement of 5HT neuronal function by caesium may be related to its ability to block K^+-channels in neuronal membranes (Kumamoto & Kuba, 1985).

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412P ELEVATED PLUS-MAZE: EFFECTS OF BUSPIRONE ON ANTINOCICEPTION AND BEHAVIOUR

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Recent findings support a link between anxiety and certain non-opioid forms of environmental analgesia (Rodgers & Shepherd, 1989). We have reported that the non-opioid analgesia observed in mice following exposure to the elevated plus-maze test of anxiety (EPM; Lee & Rodgers, 1989) is blocked by acute and chronic diazepam treatment (Lee & Rodgers, 1990). Surprisingly, however, no signficant effects of diazepam on EPM behaviour were noted. In view of the implied dissociation between analgesia and anxiety, the present study assessed the effects of the 5-HT_{1A} anxiolytic, buspirone (BSP) on analgesia and behaviour in mice exposed to the EPM. 12-14-week-old male DBA/2 mice (Bantin & Kingman, Hull) were housed in a temperature-controlled room (24±1°C) under a 12h reversed LD cycle. Testing was conducted under dim red light during the dark phase of the cycle. The EPM was based on that validated for mice by Lister(1987). Tail-flick latencies (TFL; temperature adjusted to give control values of 2-3s) were assessed by traditional radiant heat assay before (pre) and after (0, 5 & 10 min) EPM exposure. BSP HCl (Bristol Myers Co.) was dissolved in 0.9% saline which, alone, served as vehicle control; injections were performed i.p. in a volume of 10ml/kg. Mice were treated with BSP (0, 0.1, 1.0 or 10.0mg/kg) 15 min prior to brief (5 min) EPM exposure. Data were analyzed by ANOVA and appropriate follow-up tests.

Table 1 Effect of buspirone HCl on EPM analgesia. Data are given as mean TFL (s.e. mean). * $p<0.05$, ** $p<0.01$ versus pre-EPM; $^{+}$ $p<0.05$ versus vehicle control.

	Vehicle	0.1mg/kg	1.0mg/kg	10.0mg/kg BSP
Pre	2.1 (0.1)**	2.2 (0.1)*	2.3 (0.1)**	2.3 (0.1)
0	2.9 (0.2)**	2.7 (0.2)**	3.0 (0.2)*	2.2 (0.2)$^{+}$
5	2.8 (0.2)**	3.1 (0.3)**	2.7 (0.2)*	2.5 (0.2)
10	3.1 (0.4)**	2.4 (0.1)$^{+}$	2.8 (0.4)*	2.4 (0.2)$^{+}$

Consistent with previous reports (Lee & Rodgers, 1989, 1990), EPM exposure resulted in analgesia that lasted for at least 10 min ($F_{3,105}=6.39$, $p<0.01$; see Table 1). This effect was completely blocked by 10mg/kg BSP ($p<0.05$) which, unlike diazepam, also produced an anxiolytic behavioural profile in this test. Compared to vehicle controls, which showed a clear preference for closed vs open arms ($F_{2,70}=10.75$, $p<0.01$), BSP treatment signficantly attenuated closed arm preference ($F_{3,35}=4.67$, $p<0.01$). For % time spent on maze sections: vehicle, closed > open, $p<0.01$; 10mg/kg BSP, closed = open. These data suggest that serotonergic mechanisms may play a key role in EPM behaviour and analgesia.

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413P THE 5-HT_3 RECEPTOR ANTAGONIST, BRL 43694, MODIFIES BEHAVIOURAL RESPONSES TO YOHIMBINE IN THE MOUSE

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The behavioural effects elicited by 5-HT_3 receptor antagonists at extremely low dose levels, which include modification of rodent and primate behaviour in some though not all models of anxiety (File & Johnston, 1989; Jones et al., 1988), has initiated considerable pharmacological interest. The present experiments examine such effects upon the behavioural response of mice to challenge by the anxiogenic drug, yohimbine. Yohimbine induces symptoms of anxiety in man, while in rats and mice it leads to behavioural and locomotor depression coupled with an increase of sympathetic outflow (Charney et al., 1983).

The 5-HT_3 receptor antagonist, BRL 43694, was given in drinking fluid at 40 μg/l (1 μg/100g daily) for 11-16 days to adult male pair-housed DBA/2 mice. Yohimbine (2 mg/kg i.p.) was given to mice before and after the administration of BRL 43694. Control mice received injections of an equivalent volume of physiological saline at both time periods. At 40 min following injection, behaviour of each mouse during social encounters with an unfamiliar isolated resident male was recorded for a 7 min period by ethological procedures.

Yohimbine significantly decreased frequency and duration of the social element "investigate" and reduced occurrence of non-social activity, including the behavioural elements, "explore", "scan" and "dig". Yohimbine increased the duration of non-social "immobility" and the social element "crouch". Treatment with BRL 43694 significantly decreased the "immobility" induced by yohimbine and caused a significant increase above control levels in occurrence of the social elements "investigate" and "sniff". Treatment with BRL 43694 did not influence the reduction by yohimbine of the non-social activities, "explore", "scan" and "dig". Furthermore, in saline-injected mice, BRL 43694 did not significantly change frequency or duration of the social elements, "investigate" and "sniff".

The antagonism by BRL 43694 of yohimbine-induced immobility coupled with its enhancement of social investigation supports the proposal for anxiolytic properties by 5-HT_3 receptor antagonists. On the other hand, there may be an inter-relationship between 5-HT_3 receptors and α_2-adrenoceptors which is independent of anxiolytic effects. The nucleus tractus solitarius, for example, has a high density of 5-HT_3 receptor binding sites (Pratt & Bowery, 1989) and also is a site at which yohimbine affects noradrenaline release (Wemer et al.,1982). Thus, further studies are needed to interpret the significance of the present findings.

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414P ANTAGONISM OF AMPHETAMINE-INDUCED SNIFFING, BUT NOT HYPERLOCOMOTION, BY THE SELECTIVE 5-HT_3 ANTAGONIST, ONDANSETRON

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Systemic administration of (+)-amphetamine produces hyperlocomotion, suppression of grooming, and the induction of stereotyped behaviour; effects which depend upon central dopamine pathways (Robbins & Everitt, 1982). Selective 5-HT_3 receptor agonists and antagonists affect dopaminergic activity in the rat and have been shown to influence the changes in locomotion induced by (+)-amphetamine given directly to the nucleus accumbens (Costall et al., 1987; Hagan et al., 1987; Blandina et al; 1988). Hence, it is of interest to determine the degree to which selective 5-HT_3 receptor blockade influences responses induced by systemically administered amphetamine.

48 adult male rats (320-460g) were divided into six groups (N=8), and each animal was injected twice prior to a 30 min observation period; half received ondansetron (30μg.kg^{-1}) and half its vehicle (distilled water) i.p., 30 min before the test . Within each condition, animals were injected with (+)-amphetamine sulphate (1.0 or 3.0mg.kg^{-1}), or its vehicle (isotonic saline), i.p., 10 min before the observation period. During the observation period, the duration of each bout of behaviour was recorded, using a microcomputer, according to 6 response categories: locomotion, rearing, grooming, sniffing, oral activity, immobility. Subsequent microstructural analysis of these data generated the total duration, mean frequency of bouts, and mean duration of bouts, for each response category. The data were analysed by two-way ANOVA for independent groups. A p value of <0.05 was considered significant.

At 1.0mg.kg^{-1}, (+)-amphetamine increased the total duration of locomotion and sniffing, but reduced grooming ($p<0.001$ in each case). Given alone, ondansetron had no effect on these responses, but it did significantly attenuate (+)-amphetamine-induced sniffing (drug interaction term: $F_{1,28} = 8.16$, $p<0.01$). Ondansetron did not alter the effects of (+)-amphetamine on either locomotion or grooming. The larger dose of (+)-amphetamine (3.0mg.kg^{-1}) increased the total duration of locomotion, rearing and sniffing, but reduced grooming and immobility ($p<0.005$, in each case). Ondansetron had no effect on these more intense effects of (+)-amphetamine.

The main result was that ondansetron selectively antagonized the sniffing component of (+)- amphetamine-induced behavioural effects at 1.0mg.kg^{-1} i.p. The inhibition appears to be dependent both on the behavioural category and on the dose and route of (+)-amphetamine administration. This suggests that components within the syndrome of amphetamine-induced behaviour are pharmacologically dissociable.
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415P EVIDENCE FOR A PERIPHERAL COMPONENT IN THE INHIBITORY ACTIONS OF 5-HT_3 ANTAGONISTS ON DEFEAT ANALGESIA

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The onset of non-opioid defeat analgesia in male mice is potently blocked by the 5-HT_3 antagonist odansetron (Rodgers et al. 1990). The present study investigated the effects of three more 5-HT_3 antagonists on basal nociception and defeat analgesia: MDL73147 (1H-indole-3-carboxylic acid,trans-octahydro-3-oxo-2,6-methano-2H-quinolizin-8-yl ester methanesulphonate), MDL72222 (1αH,3α,5αH-tropan-3-yl-3,5-dichlorobenzoate) and MDL72699, a peripherally-acting quaternary derivative of MDL72222. Male DBA/2 mice (Bantin & Kingman, Hull) were group-housed and maintained under a reversed LD cycle in a temperature-controlled room (24±1°C). All testing was conducted under dim red light during the dark phase of the cycle. Tail-flick latencies (temperature adjusted to give 2-3 sec baselines) were recorded before drug administration, and after, drug uptake (basal) or defeat. For defeat studies, DBA/2 intruder mice were introduced into the home cage of an aggressive male BKW resident, and removed upon display of the species-typical upright submissive posture. All compounds were dissolved in 0.9% saline and administered i.p. 30 min(MDL73147), or 45 min(MDL72222 & MDL72699) before testing. Data were analysed by ANOVA and appropriate follow-up tests. MDL73147 (0.03-10 mg/kg), MDL72222 (0.01-10 mg/kg) and MDL72699 (0.01-10 mg/kg) had no significant intrinsic effects on tail-flick latency. Defeat analgesia, apparent in all vehicle-treated mice ($p<0.001$), was inhibited by MDL73147 (0.3-10 mg/kg) and MDL72222 (0.01-10 mg/kg). Similarly, the quaternary ligand MDL72699 (0.50-1.0 mg/kg) significantly attenuated the analgesic response, although with a much narrower effective dose-range (see Table 1). All drug effects were interpreted as a partial, rather than complete, blockade of the analgesic reaction to defeat experience.

TABLE 1 Effects of MDL72699 on defeat analgesia. Data are presented as Mean ±(s.e.mean) tail-flick latencies (sec). Pre=baseline, Post=post-defeat. *** $p<0.001$ ** $p<0.02$ * $p<0.05$ vs baseline; # $p<0.025$ vs vehicle.

	VEH	0.01	0.05	0.10	0.50	1.0	10.0 mg/kg
Pre	2.36(0.2)	2.36(0.2)	2.30(0.2)	2.40(0.1)	2.59(0.2)	2.38(0.1)	2.46(0.1)
Post	4.42(0.3)	5.42(0.7)	4.82(0.8)	4.36(0.5)	3.39(0.3)	3.11(0.2)	4.28(0.4)
	***	***	***	***	*	** #	***

These data are consistent with previous reports concerning an inhibition of defeat analgesia with the 5-HT_3 antagonist odansetron (Rodgers et al. 1990), and lend support to the involvement of 5-HT_3 receptor sub-types in the mediation of this form of adaptive pain inhibition. Furthermore, present findings with MDL72699, would indicate a peripheral component in the observed effects of the 5-HT_3 antagonists tested in this paradigm.

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416P SUPPRESSION OF CONDITIONED AVOIDANCE BEHAVIOUR BY BMY 14802 CANNOT BE EXPLAINED BY SIGMA OPIOID RECEPTOR ANTAGONISM

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It has recently been claimed that the novel antipsychotic profile of BMY 14802 may be attributed to antagonism at the putative sigma receptor (Taylor & Dekleva, 1987). Suppression of conditioned avoidance responding is commonly used to predict antipsychotic activity (Arnt, 1982) and in the present study we have investigated whether sigma receptor antagonism explains the action of the compound in this paradigm.

Male Swiss Webster mice (30-40g) were trained to avoid a 0.8 mA electric shock delivered through the grid floor of a two compartment shuttle box. Daily sessions of 15 trials were given, each trial consisting of a 10 sec light and tone conditioning stimulus followed by the shock (max duration 10 sec) if not avoided by crossing into the other compartment. The inter-trial interval was randomly varied from 25-40 sec. All compounds were administered subcutaneously (n=6-8), 30 min prior to testing in mice achieving an avoidance rate of >12/15 trials over the previous 2 training days. Results are expressed as the dose of compound inhibiting the maximum number of avoidances by 50%. For in vivo binding studies, mice received an i.v. injection of the prototypical sigma ligand [^{3}H]-(+)-SKF 10,047 (5 µCi per mouse) 40 min after drug administration. After a further 20 min the whole brain was homogenised in 30 vol ice cold 50 mM Tris/HCl, pH 7.4. Samples (500 µl) were taken for counting and a second set rapidly filtered through GF/B filters followed by 2 x 5 ml washes of ice cold assay buffer. The ratio of filter trapped radioactivity to that of whole homogenate was used as a measure of total in vivo binding. Specific binding was defined by subtraction of binding in animals receiving 3 mg/kg haloperidol, 1 h before death. Results are expressed as the dose of the compound reducing specific binding by 50%. Under these conditions, binding to the sigma recognition site was confirmed by the potent displacement by (+)-, but not (-)-SKF 10,047 (ED_{50} 0.25 and 15.4 mg/kg respectively) and by (+)-, but not (-)-pentazocine (ED_{50} 0.07 and 0.86 mg/kg respectively).

Compound	Conditioned avoidance	[^{3}H]-(+)-SKF 10,047 binding
	(ED_{50}, mg/kg, s.c.)	
BMY 14802	23.2	5.6
Rimcazole	>50.0	10.0
Haloperidol	0.1	0.05
Spiperone	0.08	>0.5
(+)-Butaclamol	0.15	>>10.0
(-)-Butaclamol	>30.0	>10.0

The results indicate that suppression of conditioned avoidance behaviour does not correspond with displacement of [^{3}H]-(+)-SKF-10,047 (sigma) binding and is unlikely to explain the effect of BMY 14802 in this antipsychotic model.

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417P EFFECT OF CHRONIC NICOTINE ADMINISTRATION ON STEP-THROUGH PASSIVE AVOIDANCE BEHAVIOUR IN THE RAT

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Acute post trial nicotine has dose-dependent effects on memory in rats as measured by altered entrance latency in a step-through passive avoidance (STPA) paradigm (Haroutunian et al., 1985). We have investigated the effect of chronic (-)-nicotine (nic) pretreatment in this paradigm. Male Sprague-Dawley rats (300-400 g) received nic (0.8 mg kg^{-1}, s.c.) or saline (1 ml kg^{-1}, s.c.), daily for 28 days. On the 29th day, they underwent STPA training, using apparatus similar to that of Jarvik and Kopp (1967). Animals were placed in the apparatus and step through latency was recorded. After entering the darkened compartment animals received a 1 s, 0.5 mA scrambled shock via a grid floor, 15 s later they were removed and challenged with either nic (0.8 mg kg^{-1}, s.c.) or saline (1 ml kg^{-1}, s.c.). They were placed in holding cages for 15 min and then returned to their home cages. Animals were tested 24, 72, 120 and 168 h later for entry latency without shock. Results are tabulated below as mean entry latencies (MEL) ± SEM; significance was analysed using analysis of variance with repeated measures.

Table 1: The acute effects of (-)-nicotine or saline on STPA behaviour following chronic (28 day) administration.

Treatment chronic - challenge	Animals per group	Mean entry latency (in seconds) on training day (T) or in hours after training day				
		T	24	72	120	168
Sal-Sal	12	17.3 ± 5.0	12.3 ± 1.9	8.3 ± 2.4	4.3 ± 1.1	5.2 ± 0.9
Sal-Nic	12	11.2 ± 4.2	35.3 ± 24.2	19.0 ± 13.7	14.4 ± 7.8	37.3 ± 21.1
Nic-Sal	11	22.2 ± 8.0	65.7 ± 35.1	53.0 ± 35.9	60.0 ± 35.9	35.6 ± 26.7
Nic-Nic	13	24.3 ± 8.0	59.9 ± 28.6	57.3 ± 31.1	50.6 ± 30.8	9.2 ± 4.3

There was no significant difference in MEL on the training day between animals receiving chronic nic or saline, or on the acute effects of nic in saline pretreated animals. MEL increased significantly 24-168 h after the training day in animals treated chronically with nic compared to saline and was independent of the challenge received, $F(df\ 3,132) = 3.21$, $P < 0.05$. The results suggest that chronic nic treatment alone results in enhanced retention of a STPA response.

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418P REPEATED ADMINISTRATION OF DESIPRAMINE, BUT NOT FLUVOXAMINE OR IDAZOXAN, RESULTS IN ATTENUATION OF ISOPRENALINE-INDUCED DRINKING IN RATS

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Chronic administration of many antidepressant drugs results in the down regulation of cortical beta-adrenoceptors (Banerjee *et al.* 1977). Isoprenaline causes copious drinking in rats and it is postulated that this is a central effect and that isoprenaline-induced drinking (IID) is a model of central beta-adrenoceptor status (Goldstein *et al.* 1985). We now report the effects of chronic administration of the antidepressant drugs desipramine and fluvoxamine and the alpha$_2$-adrenoceptor antagonist idazoxan, which has putative antidepressant properties, on IID in rats.

Male Wistar rats received desipramine, fluvoxamine or idazoxan (0.125mg/ml) daily for 14 days via their drinking water, an approximate daily intake of 10mg/kg/day. Following treatment the animals received isoprenaline 0.33mg/kg s.c. and fluid intake was monitored for 150 min.

Desipramine caused a significant reduction in IID, neither fluvoxamine nor idazoxan had any effect.

Table 1. Effect of chronic antidepressant treatment on IID (* P<0.05; **P<0.01)

	Mean fluid intake (ml) ± s.e.mean				
	30 min	60 min	90 min	120 min	150 min
Vehicle Control (n=6)	0.333±0.102	0.483±0.111	2.483±0.111	2.817±0.632	2.933±0.622
Desipramine (n=5)	0.060±0.024*	0.180±0.037*	0.260±0.051**	0.500±0.084**	0.600±0.084**
Fluvoxamine (n=6)	0.267±0.128	0.633±0.455	2.600±0.847	4.517±0.905	4.633±0.915
Idazoxan (n=5)	0.240±0.117	1.240±0.518	2.220±0.935	3.040±1.257	3.380±1.240

Chronic administration of desipramine 10mg/kg/day for 14 days has previously been shown to cause concurrent decrease in IID. Fluvoxamine had no effect on IID and 2-4 weeks administration of a similar dose has no effect on cortical beta-adrenoceptors (Benfield & Ward,1986). No data for the effect of chronic idazoxan treatment on central beta-adrenoceptors is available, however the results of this behavioural model suggest that there is no receptor down regulation.

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419P THE EFFECT OF CHOLECYSTOKININ (CCK) ON OXYTOCIN AND CORTISOL RELEASE IN PIGS DURING OPERANT FEEDING

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Systemic administration of CCK has been shown to decrease food intake in a number of animal species (see Ebenezer et al., 1989). Recently, Verbalis et al (1986) reported that in rats (i) i.p. injections of CCK caused increases in plasma levels of oxytocin (OT), and (ii) ingestion of food led to significant increases in plasma OT. They suggested that increased plasma OT may be viewed as a marker of the "disinclination to eat". The aim of the present study was to establish whether these findings could be replicated in pigs.

Prepubertal large white pigs (n=7, 3F, 4M, b.wt. 30 - 50 kg) were housed seperately in metal cages, and were trained to press operant panels with their snouts for food and water. Each animal was surgically prepared under halothane anaesthesia with an iv catheter in the external jugular vein. The animals were put on the following feeding schedule: At 9.15h they received a small measured meal. At 14.15h a buzzer signalled that the feeder was activated, and that the pigs could make operant responses for food. The feeder was switched off after 2h. Water was freely available. Feeding was monitored on a data logger. The pigs were injected iv with normal saline (control) or CCK (1 µg/kg) 5 min after the feeder was activated. Blood samples were collected in 10 ml heparinized tubes before (-35, -15 min) and after (5, 20, 65 min) saline or CCK injections The plasma was assayed for OT and cortisol (CORT) using radioimmunoassays previously described (see Ebenezer et al, 1989 for details).

CCK (1 µg/kg) caused a significant decrease in food intake during the 1st 20 min period after injection ($P < 0.01$). Individual pigs stopped responding on the food panel about 1 min after injection for between 3 and 18 min. Thereafter they ate normally. Analysis of the endocrine data revealed no significant changes in OT levels after CCK but significant elevations in CORT levels measured 5 min (92%, $P < 0.01$) and 25 min (63% $P < 0.05$) after injection. Futhermore, in the control experiment we found no significant increases in the levels of OT after the pigs began to eat, which suggests that OT is not secreted in response to feeding in pigs. Similar results were obtained for CORT. In contrast to findings in rats (see above) the present results show that systemic administration of CCK, or feeding per se, does not increase plasma OT in pigs. However, CCK does produce increases in plasma CORT, which indicates that the peptide induces a stressful response in these animals. Similar changes in OT and CORT have been reported in sheep after iv CCK (Ebenezer et al, 1989) and it is likely that CCK releases OT as part of a stress response. The results of this study thus suggest that increases in plasma OT may not be a good indicator of a "disinclination to eat" in species other than the rat.

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420P THE EFFECTS OF BACLOFEN ON OPERANT AND NON-OPERANT FOOD INTAKE IN RATS

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It has recently been reported that the GABA-B agonist baclofen induces feeding in satiated pigs (Ebenezer and Baldwin, 1989). In the present study we examined the effects of systemically administered baclofen on operant and non-operant food intake in rats.

Experiment 1: Male Wistar rats (280 - 350g, n=7) were trained to press a lever in an operant conditioning chamber for food reinforcement on a fixed ratio of 6 (see Ebenezer, 1985 for details). The animals were deprived of food for 22h before each session in the operant chamber. The rats were injected s.c. with either physiological saline (control) or baclofen (0.01, 0.1, 0.5 and 1.0 mg/kg) and placed seperately in the operant chamber after injection. The amount of food consumed was measured for a period of 30 min starting immediately after saline or drug administration.
Experiment 2: Male Wistar rats (n=10) that were not food deprived, were placed individually in cages in which they had free access to food and water for 90 min. The amount of food consumed was measured at 30 min intervals.Thirty min after the rats were placed in the cages, they were injected i.p. with either saline (control) or baclofen (0.125, 0.25, 0.5, 1.0 and 8.0 mg/kg).

The results obtained in the 1st experiment showed that compared with control data, baclofen 0.5 mg/kg and 1.0 mg/kg caused small but significant increases (i.e. 14.7%, $P < 0.05$, and 14.0%, $P \leq 0.05$ respectively) in food intake during the 1st 30 min after injecion. In contrast, the lower doses of baclofen did not produce any significant effects on food consumption. No overt behavioural side effects were observed in these animals at any of the doses used. In the 2nd experiment, we found that saline treated animals ate very little food (i.e. 156.3 + 82 mg in 60 min). However, baclofen 0.5 mg/kg produced significant increases ($P < 0.05$) in food intake during the 1st 30 min after administration, while baclofen 0.5 and 1.0 mg/kg produced significant increases ($P < 0.01$ and 0.05 respectively) over the total 60 min post-drug measurement period. The 8 mg/kg dose of baclofen caused the animals to go into a deep sleep-like state approximately 10 min after injection for between 3 and 4h. Baclofen had no significant effects on water intake.

In the present study we have demonstrated that low doses of systemically administered baclofen will produce short-term increases in food consumption in both hungry and satiated rats. These results extend previous findings in the pig (Ebenezer and Baldwin, 1989).

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421P IS PICA IN RATS A SENSITIVE MEASURE OF DRUG-INDUCED NAUSEA?

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Rats are unable to vomit, and other methods, e.g. conditioned taste aversion (Mackintosh, 1985), are used to assay drugs that cause nausea. Mitchell et al (1976) reported that rats that were poisoned ate soil or kaolin to reduce the physiological correlates of nausea produced by these toxic substances, and suggested that increased pica (i.e. the consumption of non-nutritive substances) may be used as a behavioural assay of noxious drug effects. In this study we investigated whether we could observe increased pica in rats treated with a dose of LiCl that is known to produce conditioned taste aversion in these animals.

Male Wistar rats (wt. 280 - 350g) were used in this study. In the 1st experiment, rats (n=10) were given 2 acclimatisation trials during which they were put seperately in cages for 30 min on 2 occasions, and had free access to food, kaolin and water. On the 2 subsequent experimental trials the animals were injected i.p. with saline (control) or LiCl (100 mg/kg), and the amount of kaolin and food consumed measured 30 and 60 min after injection. Each animal received both treatments. In the 2nd experiment, rats (n=10) that were food deprived for 22h each day, were given 2 acclimatisation trials as above, but only had access to kaolin and water. The amount of food, water and kaolin consumed was measured 30, 60 and 180 min after drug administration. In the 3rd experiment, rats (n=10) were treated as in experiment 2, except that during the 2 experimental trials the animals only had access to kaolin and water.

All rats ate small amounts of kaolin during the acclimatisation trials. In experiment 1, rats injected with LiCl did not eat any more kaolin than they did when injected with saline. In experiment 2, rats injected with saline consumed food rather than kaolin, with the most pronounced eating occuring during the 1st 30 min. In contrast, rats injected with LiCl did not eat the food or kaolin during the 1st 20 - 30 min after administration, but when they seemed to have recovered from the effects of LiCl, they consumed food in preference to kaolin. There was no significant effects on kaolin intake between the 2 treatments. In experiment 3, the rats treated with LiCl did not display any significant increases in the consumption of kaolin compared with saline treatment. The results of these experiments show that LiCl (100 mg/kg) does not cause a short-term increase in kaolin consumption in both food-deprived and non food-deprived rats. We have shown that LiCl (100 mg/kg) will produce learned taste aversion (unpublished results) and are therefore satisfied that this dose is aversive. These results thus suggest that the pica method described by Mitchell et al (1976) is not a very sensitive assay for measuring behavioural toxicity of drugs in rats.

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422P INCREASED ACTION OF THE PARTIAL INVERSE AGONIST, FG7142, DURING BARBITURATE WITHDRAWAL: PROTECTIVE EFFECT OF THE CALCIUM CHANNEL ANTAGONIST, NITRENDIPINE

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We have previously shown that calcium channel antagonists, such as nitrendipine, will protect against ethanol withdrawal (Littleton et al, 1989), benzodiazepine withdrawal (Patch et al, 1989) and nitrous oxide withdrawal (Dolin & Little, 1989). We have shown previously that there was a prolonged increase in the actions of the partial inverse benzodiazepine agonist, FG7142 after chronic benzodiazepine treatment (Little et al, 1988). We now report a similar, though shorter, effect during withdrawal from chronic barbital treatment, that was decreased by nitrendipine.

Male mice, TO strain, 30-35g, were given barbital in powdered food for seven days; 3 mg barbital per g food for two days, 4 mg/g food for two days and 5 mg/g food for three days. Controls received a matched amount of powdered food only. FG7142, was suspended in Tween 80 (0.5%) and injected i.p. at 40 mg/kg. The animals were then observed for 60 min by an observer who did not know what prior drug treatment the animals had received. Separate groups of animals were used for each experiment. FG7142 is normally only proconvulsant (Little et al, 1984), but during barbital withdrawal caused full convulsions (clonic movements of limbs and loss of posture). Convulsion incidence: 12h withdrawal 6/8; 24h withdrawal 8/8; 36h withdrawal 3/8. No convulsions were observed in control animals (0/8: $P < 0.01$ for 12h and 24h, Fisher's exact probability test). At the 24h interval, nitrendipine, 50 mg/kg i.p., suspended in Tween 80, 0.5%, 30 min before FG7142, reduced the convulsion incidence and significantly increased the latencies:-

Barbital withdrawal plus vehicle: 9/10 convulsed; latencies: 155 ± 16:
Barbital withdrawal plus nitrendipine, 50 mg/kg: 5/10 convulsed; latencies: 310 ± 57*
(*Latencies: $P < 0.01$, Student's t-test; Convulsion incidence: $P = 0.07$, Fisher's exact probability test)

Acute adminstration of nitrendipine did not alter the convulsive effects of the full inverse agonist DMCM in naive mice, and we have previously reported its lack of effect on bicuculline convulsions (Patch et al, 1989). The convulsive effects of FG7142 were increased during barbital withdrawal and this effect was decreased by the calcium channel antagonist, nitrendipine, given 30 min previously. The barbiturate withdrawal syndrome has been suggested to be due to decreased GABA transmission (Ho & Harris, 1981). The protective action of nitrendipine in the present results, and its lack of action against DMCM or bicuculline suggests that this is not the whole explanation.

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423P BENZODIAZEPINE RECEPTOR INVERSE AGONISTS IMPROVE ACQUISITION OF A MULTI-TRIAL PASSIVE AVOIDANCE TASK

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The extensive neuronal degeneration seen in Alzheimer's disease and senile dementia of the Alzheimer type, particularly in the cholinergic pathways, has been associated with the characteristic cognitive deficits of dementia. Sarter et al (1988) suggested that remaining neurones might function more effectively if the influence of inhibitory GABA neurones was attenuated by benzodiazepine receptor inverse agonists. The effect of such compounds was therefore examined in a multi-trial passive avoidance procedure based on a task shown to be sensitive to the benzodiazepine receptor ligand, ZK 93426 (Jensen et al., 1987).

The apparatus was a perspex chamber with a short, perspex-floored 'safe' side (12 x 10 cm) and a longer, grid-floored 'shock' side (16.5 x 10 cm). Mild electric footshock (100 μA, 1 s duration) could be delivered to the latter by a shock source and scrambler. Male CFLP mice (25-40 g) were dosed i.p. with a test compound 15 min before being placed on the 'safe' side. A footshock was given when the mouse first crossed to the grid side, repeated at 5 s intervals if necessary until it remained on the 'safe' side for 100 s. The number of shocks to criterion was counted. The median shocks for control groups ranged from 4 to 6, and test data are shown as a percentage of the appropriate control.

While the antagonist, Ro 15-1788, and the inverse agonist, FG 7142, had little effect, other inverse agonists significantly reduced the number of shocks to criterion. Amphetamine, well known to improve learning in avoidance tasks, was also active. Scopolamine significantly increased the number of shocks to criterion.

Table 1 Maximal effects in passive avoidance (% of control shocks to criterion)

Compound	FG 7142	Ro 15-1788	DMCM	S-135	Ro 15-4513	RU 34000	DL-Amphetamine	Scopolamine
Dose mg/kg	10	10	2	1	0.5	5	2	0.5
% control	113	83	50*	58*	60*	67*	40*	225*

* $P < 0.05$ versus control, Mann-Whitney U test, 2 tailed.

These findings suggest that a multi-trial passive avoidance test may be useful for examining the effects of inverse agonists on learning.

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424P INHIBITION OF REARING BEHAVIOUR IN RATS BY ANXIOGENIC AGENTS CORRELATES WITH THEIR ABILITY TO POTENTIATE [^{35}S]-TBPS BINDING *IN VITRO*

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In this study, we describe the ability of six putative anxiogenic agents to inhibit rearing behaviour in rats. The behaviour was measured on an elevated platform with raised sides which, for convenience, we have named the 'trough'. The effects of the six agents, in vivo, have been compared with their ability to potentiate [^{35}S] tertiary butyl bicyclophosphorothionate ([^{35}S]-TBPS) binding to the GABA-benzodiazepine receptor Cl^- channel complex in vitro; an index of the functional state of the Cl^- channel both in vitro (Gee 1988) and ex vivo (Concas et al. 1989).

Groups of male CFY rats (250-280g; n=12) were administered the following drugs intraperitoneally (i.p.), at the doses (mg/kg) and pretreatment times stated: FG7142 (1, 3, 10; 15 min); DMCM (0.03, 0.1, 0.3, 1; 10 min); β-CCM (1, 3, 10; 5 min); β-CCE (1, 3, 10; 5 min); Ro-5-4864 (2, 4, 8; 15 min); caffeine (25, 50, 75, 100; 30 min). Control animals received appropriate vehicle. Rats were placed on the 'trough', 105 cm (L) x 15cm (B) x 10cm (H); set at 70cm above the ground. Time spent rearing and the number of rears and crossings of a central white line were recorded for 3 mins. [^{35}S]-TBPS binding was measured using well washed rat cerebral cortex membranes incubated at 25°C for 120 min in Tris citrate buffer (pH 7.1), with 2nM [^{35}S]-TBPS and in the presence of 200mM NaCl and 5μM GABA. Non-specific binding was defined by 100μM picrotoxin.

FG 7142 (1-10mg/kg), DMCM (0.03-1mg/kg), β-CCM (1-10mg/kg) and Ro-5-4864 (2-8mg/kg) all produced a significant dose-related decrease in rearing time and in the number of rears and crossings ($P<0.05$, Kruskal Wallis); however, on a percentage basis the number of crossings were affected less by the treatments. β-CCE (1-10mg/kg) dose-relatedly decreased rearing time and the number of rears ($P<0.05$, Kruskal Wallis), but the number of crossings were not significantly reduced at any dose. Caffeine enhanced rearing at 25 and 50mg/kg, but at 75 and 100mg/kg significantly reduced the rearing time, and the number of rears and crossings ($P<0.05$, Kruskal Wallis). The following doses (mg/kg) reduced rearing time by approximately 50%: DMCM (0.17); β-CCM (2.8); FG7142 (3.0); β-CCE (5.4); Ro 5-4864 (6.3); caffeine (94.0). The same compounds potentiated [^{35}S]-TBPS binding with varying potencies and maxima; ranging from 23-174% above control. The pEC_{20} (-log drug conc. (M) to increase control binding above control by 20%) were: DMCM (9.0); β-CCM (8.5); β-CCE (7.4); FG7142 (6.8); Ro-5-4864 (6.1); caffeine (4.1). Correlation of pEC_{20} values with the log dose (mg/kg) giving approximately 50% inhibition of rearing was significant, ($r=0.908$, $P<0.02$).

These results suggest that, in this model, putative anxiogenic drugs inhibit rearing behaviour; this effect may be linked to an action on the GABA benzodiazepine Cl^- channel complex.

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425P EFFECT OF PRETREATMENT WITH FORSKOLIN OR 8-BROMO-CAMP ON ADENYLATE CYCLASE STIMULATION IN NG108-15 AND NCB20 CELLS

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Prostacyclin (PGI2) and adenosine A2 receptors activate adenylate cyclase in the hybrid cell lines NG108-15 and NCB20. Prolonged exposure of both cell lines to N-ethylcarboxamidoadenosine (NECA), an A2 receptor agonist, leads to a pronounced homologous desensitization of subsequent responses to A2 agonists (Keen et al., 1989). Pretreatment of both cell lines with iloprost, a prostacyclin receptor agonist, leads to loss of responsiveness to PGI2 receptor agonists; in NG108-15 cells, this homologous desensitization is accompanied by heterologous loss of responsiveness to other stimulators of adenylate cyclase, such as NECA and sodium fluoride (NaF) (Kelly et al., 1990). In this study we have investigated the role of cAMP in these desensitization phenomena by examining the effects of pretreating both cell lines with forskolin or the cell permeant cAMP analogue, 8-bromo-cAMP.

NG108-15 (passage 16-25) and NCB20 (passage 11-21) cells were grown to confluency in 80cm^2 flasks. Cells were pretreated with 10μM forksolin, 10μM 8-bromo-cAMP or the appropriate vehicle in Dulbecco's modified Eagle's medium for 2h or 17h. Cells were then harvested in phosphate buffered saline, washed three times and frozen at -80^o until required. Adenylate cyclase activity and [3H]-iloprost binding were assayed in cell homogenates as previously described (Kelly et al., 1989).

The effects of pretreatment for 17h with forskolin were apparently the same in the two cell lines. There was a significant decrease (~30%) in the maximal responses to NECA, with no change in NECA EC50 values. There was a small, but not statistically significant, decrease in the maximal responses to iloprost. This was accompanied by consistent shift to the right of the iloprost dose-response curves. Binding experiments showed a loss of ~30% of the specific binding sites for [3H]-iloprost. There was no change in responses to NaF or forskolin. The altered responsiveness seemed to be due to stimulation of adenylate cyclase by forskolin; pretreatment of both cell lines with 8-bromo-cAMP for 17h caused the same changes in responsiveness as the forskolin pretreatment. The changes seemed to be induced rather slowly, pretreatment for 2h with forskolin did not produce any change in responsiveness in either cell line.

Increased levels of intracellular cAMP led to some loss of responsiveness to PGI2 and A2 receptor agonists. There was a comparable loss of [3H]-iloprost binding sites, suggesting that this desensitization might be mediated by receptor down-regulation. The loss of receptor responsiveness is much smaller, and occurs more slowly, than the desensitization produced by pretreatment with iloprost or NECA. Increased levels of cAMP were without effect on the functioning of Gs and adenylate cyclase in these cell lines.

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426P EFFECT OF MORPHINE PRETREATMENT ON STIMULATION AND INHIBITION OF ADENYLATE CYCLASE IN NG108-15 AND NCB20 CELLS

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Prostacyclin (PGI2) and adenosine A2 receptors stimulate adenylate cyclase in the hybrid cell lines NG108-15 and NCB20; δ-opiate receptors inhibit adenylate cyclase. Pretreatment of NG108-15 cells with iloprost, a stable analogue of PGI2, leads to desensitization of responses to iloprost accompanied by heterologous loss of responsiveness to other stimulators of adenylate cyclase, such as N-ethylcarboxamidoadenosine (NECA; an A2 receptor agonist) and sodium fluoride (NaF). The inhibition of adenylate cyclase produced by morphine is potentiated following iloprost pretreatment. In NCB20 cells, iloprost pretreatment leads to desensitization to PGI2 receptor agonists, but the responsiveness of the adenylate cyclase system to other agents is unaffected (Kelly et al., 1990). In this study we have examined the effects of morphine pretreatment on subsequent responses of the adenylate cyclase system in NG108-15 and NCB20 cells.

NG108-15 (passage 16-25) and NCB20 (passage 11-21) cells were grown to confluency in 80cm^2 flasks. Cells were pretreated with 100μM morphine or vehicle in Dulbecco's modified Eagle's medium for 24h, then harvested in phosphate buffered saline, washed twice and frozen at -80^{o}C until required. Adenylate cyclase activity and [3H]-iloprost binding were assayed in cell homogenates as previously described (Kelly et al., 1989).

Pretreatment of NG108-15 cells with morphine led to an increase in the basal activity of adenylate cyclase by ~50% and, as found by Sharma et al. (1977), there was a potentiation of the receptor mediated stimulation of adenylate cyclase by iloprost and NECA. The maximal response to iloprost was increased by ~25%; the maximal response to NECA was increased by ~35%. There was no apparent change in EC50 value for either agonist. Morphine pretreatment had no effect on the stimulation of adenylate cyclase by NaF, or on the inhibition of adenylate cyclase by morphine itself. Binding studies with [3H]-iloprost showed that there was no change in the number of PGI2 receptors in morphine treated NG108 cells. In NCB20 cells, morphine pretreatment had no effect on basal adenylate cyclase activity or on responses to iloprost, NECA, NaF or morphine.

There is a fundamental difference in the effects of morphine pretreatment in the two related hybrid cell lines NG108-15 and NCB20. The molecular mechanism underlying the potentiation of agonist stimulation of adenylate cyclase in NG108-15 cells is at present unclear. The increase in basal activity and responses to stimulatory agonists cannot be due to a loss of functional Gi or an increase in Gs activity as dose/response curves to morphine and NaF are unaffected. Furthermore, there is no increase in the number of stimulatory receptors. These effects of morphine may represent a novel mechanism by which receptor responsiveness is regulated.

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427P NICOTINIC BUT NOT MUSCARINIC RECEPTOR AGONISTS DEPOLARIZE SH-SY5Y HUMAN NEUROBLASTOMA CELLS

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Activation of muscarinic receptors expressed on SH-SY5Y human neuroblastoma cells produces a rapid and marked increase in inositol(1,4,5)trisphosphate accumulation and a biphasic increase in $[Ca^{2+}]_i$. The observed $[Ca^{2+}]_i$ response involves the release of stored Ca^{2+} and Ca^{2+} influx (Lambert & Nahorski 1990). Detailed knowledge of the voltage and agonist gated currents in these cells is lacking although previously we have reported that calcium influx occurs through a dihydropyridine and conotoxin-insensitive mechanism (Lambert & Nahorski 1989). Here we present complementary second messenger and electrophysiological evidence concerning voltage-sensitive and cholinergic agonist-gated currents in SH-SY5Y cells.

$[Ca^{2+}]_i$ measurements were made in Fura 2-loaded cells and calculated according to Grynkiewicz et al. (1985). Inositol phosphate (InsP) production was measured in cells prelabelled with [^{3}H]inositol and subsequently test-stimulated in [^{3}H]inositol and 5mM Li^+ for 30 mins. Whole cell patch recordings were made from single perfused cells using an Axopatch amplifier, drugs were applied by pressure ejection from a glass pipette. All data are mean ± SEM (n=3-5).

Carbachol and muscarine caused a dose-related increase in InsP production (EC_{50} 13.7 ± 2.4μM and 8.3 ± 1.1μM respectively). Nicotine (1mM) was ineffective. Both carbachol and muscarine (100μM) induced a biphasic increase in $[Ca^{2+}]_i$. For muscarine $[Ca^{2+}]_i$ rose from a basal of 97 ± 16 nM to a peak of 487 ± 17 nM at 11 sec, before declining to a plateau of 265 ± 7nM. Nicotine (1mM) was ineffective. In whole-cell patch recordings both carbachol and nicotine (1mM), but not muscarine, produced a fast inward current at negative holding potentials (0.5 nA at -60 mV). Single applications of agonist were followed by prolonged desensitization. At positive potentials little or no outward current was observed. The reversal potential was estimated to be +8.1 ± 1.5 mV. These properties are similar to those observed for neuronal nicotinic receptors (Mathie et al. 1987). Voltage clamp experiments demonstrated that these cells possess a fast transient inward current which is tetrodotoxin sensitive and a sustained outward current which was blocked by external tetraethylamonium or by internal caesium ions.

These results indicate that a voltage sensitive sodium current and a delayed rectifier potassium current are present, but no evidence was found for a transient outward or 'A' current. In addition nicotine induced a transient inward current, or depolarization, while muscarine had no detectable effect. The converse is true for the effect of these agonists on InsP levels and $[Ca^{2+}]_i$. These results suggest that muscarinic receptor-mediated Ca^{2+} influx is not due to direct activation of voltage-sensitive Ca^{2+} channels.

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428P EFFECT OF ATRACURIUM ON TWITCH RELAXATION RATE IN THE RAT DIAPHRAGM PREPARATION

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Muscle relaxation rate (RR), following twitch or tetanic stimulation, has been used as an indicator of a skeletal muscle fatigue or durability, in both man and animal species (Edwards,Hill & Jones,1972;1975; Koulouris,Vianna,Mulvey,Green & Moxham,1989; Moxham,Morris,Spiro,Edwards & Green,1981; Effithimiou,Bellman, Holman,Edwards & Spiro,1986). In the present investigation, twitch relaxation rate (RR) was analysed in the rat phrenic nerve-diaphragm preparation, and the effect of a relatively new skeletal muscle relaxant, atracurium (Stenlake,1979),on the RR was studied in this preparation.

Eight, adult, male, Sprague-Dawley rats, were killed,by a blow to the head and bled. The phrenic nerve-diaphragm preparation was dissected and set up in an organ bath containing 80 m l of Krebs-Henseleit solution bubbled with 5% CO_2 in O_2. The phrenic nerve was stimulated at 0.2 Hz or 1 Hz, with 5V, supramaximal, and 0.2 ms pulse duration. The contractile responses produced by nerve or muscle stimulation were recorded isometrically. Muscle relaxation rate (RR) was measured and expressed in terms of the $t_{\frac{1}{2}}$ (in ms),i.e. the half-time for relaxation was measured from the peak twitch tension (also see Edwards,et.al.,1972), assuming relaxation phase followed an exponential time course (Edwards,et.al.,1972;1975).

The results are shown in Table 1.

Table 1. Effect of atracurium (1 μM) on twitch relaxation rate in the rat isolated diaphragm preparation.

Twitch	Peak (g)	Time to Peak ms	Time to $t_{\frac{1}{2}}$ ms
Control	2.86±0.05	38.63± 1.65	31.75± 1.50
Atracurium	1.41±0.03	51.00± 9.11	20.75± 1.39
% Change	50.00±15.1	31.91± 18.98	34.61± 9.60
P <	0.001	0.05	0.01

The results are means± s.e.,n=8 rats. The RR (or $t_{\frac{1}{2}}$ ms) obtained in the present study are similar to those reported by other workers in other preparations (excluding diaphragm of the rat). Atracurium reduced the peak, increased the time to peak and decreased the $t_{\frac{1}{2}}$, significantly, in the rat diaphragm preparation.

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429P EFFECTS OF MYO-INOSITOL AND D-MYO-INOSITOL 1,2,6-TRISPHOSPHATE (PP56) ON NEURONAL DYSFUNCTION IN STREPTOZOTOCIN-DIABETIC RATS

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Diabetic neuropathy is characterised by reduced nerve conduction velocity and an increased resistance to ischaemic blockade of action potential generation. Both phenomena may be observed in rats with experimental diabetes mellitus, where they may be related to depleted nerve myo-inositol levels. The present study was designed to test this association, by feeding diabetic rats myo-inositol to prevent the depletion, and to examine the effects of treatment with myo-inositol 1,2,6-trisphosphate (PP56; M.J.Sirén, 1984, U.S. Patent No. 4735936; Perstorp Pharma, Sweden). The latter was included because previous work had shown an attenuation of deficient motor nerve conduction velocity (MNCV) in treated diabetic rats. Thirty male Wistar rats made diabetic with streptozotocin (50 mg/kg i.p.) formed 3 groups - untreated, PP56 treated (1mg/h s.c. by Alzet osmotic pump 2ML4) and myo-inositol treated (500 mg/rat/day via drinking water); 10 non-diabetic rats formed a control group. Treatments were maintained for 3 weeks. MNCV was then measured under halothane anaesthesia in the sciatic/tibialis system; 48-72 h later rats were killed by a blow to the head and both sciatic nerves removed. One was desheathed and prepared for recording compound action potentials *in vitro* (maintained in Krebs-Henseleit bicarbonate buffered saline containing 0.5 mM myo-inositol). Preparations were gassed initially with 95% O_2/5% CO_2, followed by a 40 min period during which the O_2 content was reduced to 8% (balance N_2), to study the effect of this period of hypoxia on compound nerve action potential amplitude. MNCV (m/sec ± SEM) in diabetic rats (45.3±1.2) was slowed compared to controls (57.7±1.6; $p<0.05$ by one-way ANOVA). This slowing was attenuated by PP56 (53.4±2.0) and by myo-inositol (50.0±1.5), both values being significantly greater than untreated diabetics ($p<0.05$ by ANOVA). The decline in action potential amplitude (% of initial value ± SEM) during the 40 min period of hypoxia was greater in controls (54.8±4.9) than in diabetics (76.1±3.0; $p<0.01$). Declines were similar in both treated groups (PP56 - 69.4±5.1 [$p<0.05$ vs. controls]; myo-inositol - 79.9±5.2 [$p<0.01$ vs. controls]). This implicates different mechanisms in the development of these two diabetes-associated abnormalities of nerve function.

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430P ALTERED NEUROPEPTIDE GENE EXPRESSION IN EXPERIMENTAL DIABETES

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Deficits in the amount of critical components of axoplasm undergoing axonal transport may contribute to the aetiology of diabetic distal polyneuropathy. The present study was designed to examine the neuronal handling of substance P in rats with experimental diabetes mellitus. Further studies were performed to determine whether altered levels of substance P in diabetic rats could be explained by changes in gene expression of the precursor protein, preprotachykinin (PPT). Diabetes was induced with streptozotocin (50 mg/kg i.p.) and rats were studied 4 weeks later. Dorsal root (L_4 and L_5) and trigeminal ganglia were removed rapidly at death. Ganglia from pairs of rats were pooled and homogenised in guanidine isothiocyanate (10%) at pH 7.0. DNA and proteins were removed by extraction with phenol/chloroform, leaving the RNA in the aqueous phase. The RNA species were separated by Northern gel electrophoresis and transferred to a nylon membrane by electroblotting. A [^{32}P]-labelled 39-mer oligonucleotide probe complimentary to PPT was then hybridised to the membrane. The resultant hybrids were revealed by autoradiography. Irides and 1 cm portions of sciatic nerve were removed from the same rats at death, snap frozen in liquid N_2 and substance P extracted (< 2h later) in boiling 2M acetic acid containing 10mM HCl, 1 mM EDTA and 1 mM dithiothreitol. Substance P-like immunoreactivity (SPLI) was measured by radioimmunoassay, as described elsewhere (Robinson *et al.*, 1987). Sciatic nerve from diabetic rats contained much less SPLI (46.8±5.6 [sem] pg/cm) than segments from control rats (87.4±12.5 [sem] pg/cm; $p < 0.01$). This was associated with markedly reduced levels of the PPT mRNA (relative to total RNA) in the dorsal root ganglia of the diabetic rats compared to control rats. In contrast the irides of the diabetic rats contained significantly more SPLI (182.7±35.5 [sem] versus 64.9±9.2 [sem] pg/iris; $p < 0.01$). This change was associated with increased PPT gene expression (relative to total RNA) in the trigeminal ganglia of the diabetic animals. These findings indicate fundamental changes in gene expression and translation for substance P in experimental diabetes. We have no explanation for the dichotomy of changes in the two systems studied.

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431P CLONIDINE INHIBITS ^{45}Ca ENTRY INTO SYNAPTOSOMES AND, IN ADDITION, REDUCES EXCHANGEABLE INTRACELLULAR Ca^{2+} POOLS.

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Presynaptic α_2-adrenoceptor activation decreases intrasynaptosomal free Ca^{2+}($[Ca^{2+}]_i$) by inhibiting Ca^{2+} entry through N type Ca^{2+} channels(Xiang et al.,1989). In this study, we have examined the effects of α_2-adrenoceptor activation on $[Ca^{2+}]_i$ and ^{45}Ca accumulation in rat cortical synaptosomes(Dunkley et al.,1988) under normal and depolarised conditions and also, at various extracellular Ca^{2+} concentrations($[Ca^{2+}]_o$).

^{45}Ca accumulation into synaptosomes was voltage dependent and, at 5mM $[K^+]_o$, was 7nmole/mg protein/2min, indicating that some Ca^{2+} channels are operational under resting conditions. Although clonidine(1μM) had no effect in hyperpolarised(0.1-1mM $[K^+]_o$) synaptosomes, it significantly reduced rapid(2min) ^{45}Ca accumulation under polarised(5mM $[K^+]_o$) and depolarised(10-60mM $[K^+]_o$) conditions. The % decrease in ^{45}Ca accumulation was relatively constant(30%) from 5-60mM $[K^+]_o$, confirming that clonidine acts via N type (less depolarisation-dependent) but not L type Ca^{2+} channels. The extent to which α_2-adrenoceptors and N type Ca^{2+} channels co-exist or the extent to which α_2-adrenoceptor activation in vivo can close N type Ca^{2+} channels is uncertain. However, on the basis of our data which showed that clonidine could reduce ^{45}Ca accumulation by as much as 50%, it is clear that a large fraction of N type Ca^{2+} channels can be blocked by α_2-adrenoceptor activation. This indicates that either α_2-adrenoceptors are widespread or alternatively, that they have a very large effect on N type Ca^{2+} channels on specific neurones.

When the clonidine-mediated decreases in $[Ca^{2+}]_i$ and in ^{45}Ca accumulation were measured as a function of $[Ca^{2+}]_o$, the reductions in both ^{45}Ca accumulation (24%, $P<0.01$) and $[Ca^{2+}]_i$(26%, $P<0.01$) were most apparent at 1mM $[Ca^{2+}]_o$. They were absent at lower than 0.8mM $[Ca^{2+}]_o$ and were attenuated at 2-5mM $[Ca^{2+}]_o$. These data show that the inhibitory effects of clonidine on both $[Ca^{2+}]_i$ and ^{45}Ca accumulation are maximal under physiological conditions, but that the decrease in $[Ca^{2+}]_i$ is much smaller (0.0006% of that in ^{45}Ca accumulation).

^{45}Ca accumulation reached equilibrium after 5min and specific activity measurements showed that after 5min incubation, only 10% of the total pool was exchangeable and that this fraction was reduced by 23%($P<0.01$) in the presence of clonidine. We conclude that clonidine 1) reduces the rate of influx of Ca^{2+} and 2) reduces the size of the rapidly exchangeable Ca^{2+} pool.

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432P COMPARATIVE EFFECTS OF PANCURONIUM AND VECURONIUM ON RAT ISOLATED DIAPHRAGM AND KIDNEY

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The aim of the present investigation was to study the comparative effects of pancuronium and vecuronium on diaphragmatic twitch contraction and on cell membrane and structure in both the diaphragm and kidney tissues and to relate neuromuscular effects to cellular effects of these muscle relaxants at both low and high concentrations.

12 male, adult, Sprague-Dawley rats were killed by a blow to the head and bled. The tissues were removed and placed in Krebs-Henseleit solution. The effects of the two muscle relaxants on indirectly-elicited twitch tension (at 0.2 Hz) were studied using organ bath preparation. The cellular effects of these drugs were assessed histologically (Nigrovic, Klaunig, Smith, Schultz & Wajskol, 1986; Makinde & Wali, 1988; Makinde, Suer, Altinel & Wali, 1988). The results are shown in Table 1.

	Concentration	Twitch blockade (%)	Cellular effects on the diaphragm and kidney tissues
Pancuronium	low (350 nM)	64± 1.9% **	No significant effect on cell membrane or structure in either type of tissues.
	high (35 μM)	100% blockade	Profound lesions in the cell membrnes, dissolution of organelles, fragmentation of kidney tubules and lymphocyte infiltration. No significant effect on the diaphragmatic cells.
Vecuronium	low (320 nM)	39±2.3% *	No apparent effect on cell membrane or structure in both types of tissues.
	high (32 μM)	88± 1.5%**	Only a slight distortion of cellular organisation. No apparent damage to cell membrane or structure.

The results are means±s.e.,n=12 rats, *$p<0.05$, **$p<0.01$. It was concluded that pancuronium was a more powerful muscle relaxant than vecuronium. In addition, at high blocking concentrations, only pancuronium produced lesions in the membrane, fragmentation of kidney tubules and inflammatory responses.

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433P AMELIORATION OF CISPLATIN-INDUCED ACUTE RENAL FAILURE IN THE RAT WITH 8-CYCLOPENTYL-1,3-DIPROPYLXANTHINE

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Aminophylline has been shown to reduce the nephrotoxic effects of cisplatin in the rat (Heidemann *et al* 1989). This beneficial effect of aminophylline was attributed to adenosine antagonism. We have shown that 8-cyclopentyl-1,3-dipropylxanthine (CPX) is a selective A_1 adenosine antagonist *in vivo* and can ameliorate glycerol-induced acute renal failure (ARF) in the rat (Kellett *et al* 1989). In this study the effect of CPX on the development of cisplatin-induced ARF has been evaluated. Male Wistar rats were injected with cisplatin (6 mg kg^{-1} i.v.) and immediately treated with CPX (0.1 mg kg^{-1} i.v.) or vehicle (1.0 ml kg^{-1} i.v.; 1.0% DMSO, 0.75% 1M NaOH in saline). CPX or vehicle treatment was repeated 12, 24 and 36h later. One group of rats was injected with cisplatin and received no treatment. Renal function was assessed by plasma urea and creatinine levels, 24h urine output and clearances of [^{3}H]-inulin (C-IN) and [^{14}C]-p-aminohippurate (C-PAH).

TABLE 1

	Plasma Urea (mg dl^{-1})		Plasma Creatinine (mg dl^{-1})		C-IN (ml min^{-1} $100g^{-1}$)	C-PAH (ml min^{-1} $100g^{-1}$)
	Day 0	Day 4	Day 0	Day 4	Day 8	Day 8
Group 1 No treatment	33 ± 2	126 ± 12	0.60 ± 0.06	2.03 ± 0.28	0.47 ± 0.05	1.06 ± 0.18
Group 2 Vehicle treated	43 ± 3	152 ± 10	0.61 ± 0.07	2.63 ± 0.31	0.38 + 0.06	0.63 ± 0.10
Group 3 CPX treated	35 ± 2	84 ± 9*┤	0.60 ± 0.05	1.05 ± 0.13*┤	0.83 ± 0.09**┤	1.69 ± 0.27┤

Mean ± s.e. mean (n = 15); * $P < 0.05$; ** $P < 0.01$ relative to group 1; ┤ $P < 0.01$ relative to group 2 (ANOVA).

All rats in the CPX and vehicle treated groups survived to Day 8, but mortality in the untreated group was 20%. Rats treated with CPX had significantly lower urea and creatinine levels compared to untreated and vehicle treated animals. 24h urine output increased 4 fold on Day 4 following cisplatin injection (32 ± 4 ml) compared to control values (8 ± 1 ml). This polyuria was unaffected by either CPX or vehicle treatment. C-IN and C-PAH of CPX treated rats were significantly higher than in vehicle treated rats. These results provide further evidence that adenosine is involved in cisplatin-induced ARF and show that A_1 selective adenosine antagonists can limit the renal impairment produced by this anti-cancer agent.

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434P TRANSEPITHELIAL WATER MOVEMENT IN RESPONSE TO ARGININE-VASOPRESSIN, CARBAMEZAPINE, CHLORPROPRAMIDE AND DEMECLOCYCLINE IN TOAD (*BUFO MARINUS*) URINARY BLADDER

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Carbamazepine (CBZ) is known to cause water retention and hyponatraemia in man but its precise site and mechanism of action is disputed (Stephens et al, 1977; Fraham et al, 1969). Toad urinary bladder serves as an excellent model epithelium for investigation of salt and water homeostasis (MacKnight et al, 1980). Our aim was to establish whether CBZ has biological activity in the toad urinary bladder and, if so, whether this activity is mediated by anti-diuretic hormone (ADH). We used a simple gravimetric technique (Bentley, 1958), which is highly sensitive to arginine-vasopressin (AVP), to assess transepithelial water movement in the isolated urinary hemibladder. The validity of this technique as a pharmacological tool was confirmed using chlorpropamide (CPM) and demeclocycline (DMC). CPM enhanced the response to AVP (50 nM) in a dose dependent manner, and a dose of 500 μM CPM increased the AVP response by 59 $\pm$ 9% (n = 6, $p<0.001$). DMC (500 μM) reduced the response to AVP (50 nM) by 37 $\pm$ 6%, (n = 6, $p<0.001$). CBZ had no anti-diuretic activity of its own in the dose range 1-100 μM but, at a dose of 25 μM, reduced the effect of AVP (50 nM) by 42 $\pm$ 3% (n = 6, $p<0.001$). These in vitro data for CBZ are at odds with earlier findings in vivo and do not support the proposal that the drug exerts its effect primarily upon the renal tubule, either directly or by interaction with AVP. The mechanism by which CBZ exerts its anti-diuretic activity in man remains unclear.

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435P INTERACTION OF ANGIOTENSIN II AND PERFUSION PRESSURE ON RENAL FUNCTION IN THE 2K-1C GOLDBLATT HYPERTENSIVE RAT

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In early phase of 2K-1C Goldblatt hypertension, Rostand and Kirk (1984) reported attenuation of diuresis and natriuresis in the isolated perfused non-clipped kidney in response to increases in perfusion pressure. Indirectly, the in vivo studies (4 weeks post-clipped) of Ding et al (1988) utilising cilazapril, indicated that this attentuation was mediated by AII. In this study, vasopressor doses of AII were employed to investigate the tubular function of the non-clipped kidney with and without changes in renal perfusion pressure. Clips (0.25mm diam) were applied to the right renal arteries of rats (170-200g) and 4-5 weeks later left renal function measured during AII infusion with ACE inhibition (Al-Qattan & Johns, 1989).

Table 1: Absolute differences in response to AII infusions

	Uncontrolled RPP (n=8)				Controlled RPP (n=8)			
	BV	20	45	150	BV	20	45	150
BP mmHg	140±5	16±2*	30±4*	42±2*	158±7	36±5*	45±6*	47±4*
RPP mmHg	136±6	17±2*	32±3*	41±2*	153±6	1.8±1	1.3±2	1.2±2
RBF ml/min/kg	14±1	-3.5±3*	-5.1±.8*	-4.7±3*	22.8±2	-7.8±.7*	-9.2±.5*	-11.6±1.4
GFR ml/min/kg	2.1±.2	-.23±.06*	-.00±.09	.02±.08	3.6±.2	-.06±.3	-.4±.2	-0.74±.2
UF µl/min/kg	99±16	26±16	67±9*	116±13*	83.1±4	-29±4*	-37±8*	-50±7*
FENa %	5.7±1	2.4±1.3	4.4±.7*	6.6±.5*	2.7±.5	-.07±.1	-1.3±.3*	-1.6±.3*

BP = blood pressure. RPP = renal perfusion pressure. RBF = renal blood flow. FENa = fractional excretion of sodium. BV = basal values. GFR = glomerular filtration rate. UF = urine flow (AII; 20, 45 or 150 $ng.Kg^{-1}.min^{-1}$).

Compared to a normotensive kidney (Al-Qattan & Johns, 1989), the non-clipped kidney showed almost double the increase in sodium and urine output in response to similar rises in perfusion pressure. However, the percentage differences for both were similar suggesting no shift to the right in the non-clipped kidney function. When RPP was controlled similar levels of antidiuresis and antinatriuresis were found implying no differences between the tubular sensitivities of the two types of kidney to AII. The unchanged response to pressure of the non-clipped kidney might be a normal physiological response to escape from sodium and water retention induced by the high circulating levels of AII as suggested by Hall et al (1984).

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436P ISOLATION OF TUBULE SEGMENTS RICH IN ARGININE-VASOPRESSIN (AVP) RECEPTORS

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The action of the antidiuretic hormone, arginine-vasopressin (AVP), is central to the regulation of body fluid tonicity and water homeostasis. In rats, AVP receptors are found in abundance in the medullary portion of the thick ascending limb (MTAL) (Imbert-Teboul et al., 1978) and the collecting tubule (CT) (Berliner & Bennett, 1967). We have developed a rapid and simple method of isolating these tubular segments from rat kidneys.

Kidneys are removed from male Wistar rats (200-350g body weight) and cut into thin slices along the cortico-papillary axis. Slices are placed in ice-cold phosphate-buffered saline (PBS), pH 7.3 - 7.4. Under a dissecting microscope the inner stripe of the outer medulla is excised from each slice. The inner stripe comprises segments of MTAL, CT and thin descending limb (TDL). These fragments are forced through 250 um, 150 um, and 100 um stainless steel sieves in succession. The sieves are held by a Bellco tissue collector. The tubule segments are washed through with PBS at each stage. The suspension is then poured onto a 20 um nylon sieve, which retains 70-80% of the MTAL and CT segments, whilst allowing passage of blood cells, debris and TL segments. The sieve is then turned over and the MTAL and CT segments collected by rinsing the sieve with 15 ml ice-cold PBS spread with a syringe (with 25g needle) with mild pressure.

The suspension is concentrated using an Amicon Centriprep 10 concentrator which is subjected to two 10 minute spins at 4000 rpm (1400 x g) in a bench centrifuge. This concentrates the suspension four to five fold. The final volume of the purified suspension is 3 ml. The tubule suspension prepared contains almost exclusively MTAL and CT segments. A few TDL segments are recognised as occasional contaminants. Light microscopy and slide photographs taken show the tubule segments to be intact. Preliminary radioreceptor studies with radioactive AVP-I^{125} indicate the viability of the tubule segments collected. This technique provides up to 290 ug protein from 6 rat kidneys in 3 hours.

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437P EFFECTS OF BENOXAPROFEN ON MYELOPEROXIDASE (MPO) ACTIVITY IN ISCHAEMIC/REPERFUSED RAT KIDNEYS

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In a recent study using purified human neutrophils it was concluded that neutrophils contribute to ischaemia/reperfusion injury in the isolated rat kidney (Linas et al, 1988). In the light of this finding we have developed an in vivo model to determine the effects of various agents on renal ischaemia/reperfusion injury. Ischaemia/reperfusion damage was induced by a method based on that used by Paller et al (1984).

Male AP1 rats were anaesthetised, placed onto a blanket at 37°C and both kidneys exteriorised. The renal artery, vein and ureter of the left kidney were clamped for 1 h and the animals were covered with another blanket to maintain body temperature. After 1 h the kidneys were reperfused, replaced into the body cavity and the animals allowed to recover. Following various periods of reperfusion (0.25- 48 h) both kidneys were removed, washed in saline, frozen in liquid nitrogen and stored at -5°C.

Each kidney was defrosted, weighed and assayed for the neutrophil specific enzyme MPO by a method based on that of Bradley et al (1982). In control unclamped kidneys the highest level obtained for MPO was 2.34 ± 1.1 units/g tissue (n = 6). The reperfused kidneys (3-48 h) showed a significant time related elevation in MPO activity from 17.7 ± 4.5 units/g at 3 h (n = 8) up to a peak of 38.1 ± 7.8 units/g (n = 8) at 24 h. MPO activity then declined to 11.3 ± 2.9 units/g (n = 9) by 48 h.

In later experiments, where benoxaprofen was administered, only the left kidney was exteriorised prior to clamping. Reperfusion was for 6 or 24 h and animals received either drug or water pre-treatment.

Benoxaprofen 10 mg/kg p.o. (-48 h, -24 h and -1 h prior ischaemia) reduced MPO activity to 14.4 ± 6.0 units/g (n = 7) compared to the control group (45.1 ± 4.6 units/g, n = 9) following 6 h reperfusion. When this study was repeated using a 24 h reperfusion period benoxaprofen pre-treatment failed to reduce MPO activity in reperfused kidneys (24.7 ± 4.4 units/g, n = 9) as compared to controls (32.4 ± 5.5 units/g, n = 9).

It is concluded that, using a homologous system, neutrophil infiltration is an important aspect of ischaemia/reperfusion damage in the rat kidney.

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438P FAILURE OF THE ATP-DEPENDENT K+ CHANNEL INHIBITOR, GLIBENCLAMIDE, TO REDUCE REPERFUSION-INDUCED OR ISCHAEMIC ARRHYTHMIAS IN RAT HEARTS

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ATP-dependent K^+ channels have been demonstrated in isolated cardiac myocytes and papillary muscles (Sanguinetti et al, 1988). These channels are normally closed but open when intracellular ATP levels fall, as in the case of hypoxia or ischaemia (Noma, 1983). The opening of these channels results in the rapid efflux of K^+ from myocytes and a reduction in action potential duration and, hence, effective refractory period (Sanguinetti et al, 1988). Such a situation would lead to a dispersion in refractoriness and would be pro-arrhythmic. To clarify whether these channels play an important role in the genesis of arrhythmias in rat hearts, we have investigated the effect of the ATP-dependent K^+ channel inhibitor, glibenclamide, on ischaemic and reperfusion-induced arrhythmias in vitro and in vivo.

In the initial study, isolated Langendorff-perfused rat hearts were subjected either to coronary artery occlusion (CAO) for 30 min, or 15 min CAO followed by 5 min reperfusion. Glibenclamide (0.1-10 µM, n = 10) had no significant effect on the incidence or duration of ventricular fibrillation (VF) and ventricular tachycardia (VT) during ischaemia or reperfusion. In contrast, the Class I antiarrhythmic agent, lignocaine (10 µM, n = 10), significantly reduced the incidence of reperfusion-induced VF from 100% to 10% ($p<0.001$).

For the in vivo study, an anaesthetised rat preparation with transient (7 min) CAO followed by reperfusion (5 min) was used as described previously (Crome et al, 1986) and the rhythm disturbances were analysed according to the Lambeth Convention (Walker et al, 1988). Glibenclamide or vehicle control (DMSO) were administered intravenously 10 min prior to CAO. Glibenclamide (1 and 10 mg/kg, n = 10) reduced the incidence of VF from 60% (control) to 30% and 30%, VT from 90% (control) to 80% and 80% and mortality from 50% (control) to 30% and 20%, respectively. However, these reductions did not reach a level of statistical significance. Thus, glibenclamide afforded little or no protection against ischaemia-induced or reperfusion-induced arrhythmias in rat hearts. This suggests that the increased K^+ efflux (via ATP-dependent K^+ channels) within the rat myocardial cells does not appear to play an important role in the genesis of ventricular arrhythmias as a result of ischaemia or reperfusion.

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439P INVESTIGATION OF THE BRADYCARDIC EFFECTS OF PROPOFOL ON THE GUINEA-PIG ISOLATED RIGHT ATRIUM

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The intravenous anaesthetic Diprivan (propofol) has been previously reported to cause a bradycardia in guinea-pig isolated atria (Briggs et al 1989). Moreover, recently, Bunting et al (1989) have demonstrated that Diprivan, etomidate and thiopentone produce negative chronotopic effects in rat isolated atria and that these compounds may act as weak Ca^{++} antagonists in cardiac muscle. Moreover, it has been further suggested that some general anaesthetics might interfere with Na-Ca exchange at the sarcolemma leading to cardio-depression (Haworth et al 1989). It was decided, therefore, to investigate the effects of Diprivan on guinea-pig isolated right atria in some detail.

Right atria were set up on platinum recording electrodes immersed in Krebs solution at 37°C, gassed with 95% O_2/5% CO_2. The electrodes were linked to a modular cardiotachometer interfaced with a computer which performed the necessary data analysis. Diprivan was cumulatively added at 0.01, 0.1 and 1.0mM, allowing 15min between each dose to attain equilibrium. Putative antagonists, atropine, PD116948, glibenclamide (all 1uM) and bicucculine (10uM) were added to the tissues and left in contact for 60min prior to addition of Diprivan. In addition, the effects of excess $CaCl_2$ (Ca^{++}ions 10mM) on the bradycardic effects of Diprivan (0.1mM) and carbachol (0.1uM) were evaluated. Results were expressed as changes in absolute rates or calculated as a pecentage decrease in rate compared to the resting rates.

Diprivan at 0.01, 0.1 and 1.0mM produced graded decreases in the spontaneous rate (9.1±2.4, 39.0±5.0 & 71.5±13.1% respectively, n=4-8). In all subsequent experiments, 0.1mM Diprivan was employed. Antagonists of muscarinic, adenosine A1, and GABA receceptors and ATP-sensitive K^+-channels were all ineffective against Diprivan-induced bradycardia. Addition of Ca^{++}ions to tissues pre-treated with Diprivan (0.1mM) led to a recovery in the rate (rate prior to Diprivan 206.0±11.0, after Diprivan 94.2±20.8; after Ca^{++}ions 179.0±10.2 bts/min). No recovery was observed in tissues which had previously been treated with carbachol (0.1uM) (rate prior to carbachol 195.8±4.1, after carbachol 132.7±5.2; after Ca^{++}ions 127.7±14.9).

The concentration-related bradycardia produced by Diprivan did not appear to be mediated via muscarinic, adenosine A1, and GABA receptors or ATP-sensitive K^+-channels. It did appear, however, that the bradycardia was sensitive to the Ca^{++}ion concentration and it is possible that Diprivan might act as a weak Ca^{++} antagonist in cardiac muscle in vitro.

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440P EFFECT OF FLOSEQUINAN ON ISCHAEMIC ARRHYTHMIAS IN ANAESTHETISED RATS

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Flosequinan is currently under clinical development for the treatment of heart failure, a condition associated with rhythm disturbances. To determine whether flosequinan has either proarrhythmic or antiarrhythmic activity it has been tested in a model of ischaemia-induced arrhythmias in anaesthetised rats.

Male Charles River rats (300-400g) were anaesthetised with pentobarbitone (60 mg/kg ip), and were prepared for coronary artery ligation by the method described by Clark et al (1980). Drugs or saline were given intravenously and the coronary ligature permanently tied 5 min later. Ischaemically-induced arrhythmias were monitored over a 30 min post-ligation period. The mortality rate, total number of premature ventricular beats (PVB) and incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) are shown in table 1.

Table 1 Effect of various drugs on % mortality and PVB score (mean ± s.e.mean) and % incidence of VT and VF in survivors in coronary ligated anaesthetised rats

	Number of animals used	Mortality due to VF %	Survivors			
			n	PVB	% incidence of VT	% incidence of VF
Saline 1 ml/kg	61	25	46	638 ± 84	91	35
Flosequinan 6.0 mg/kg	12	33	8	679 ± 196	88	50
Milrinone 0.3 mg/kg	13	23	10	767 ± 211	90	20
Disopyramide 10.0 mg/kg	7	0	7	177 ± 164*	14*	0*
Isoprenaline 0.1 µg/kg/min	11	73*	3	370 ± 140	100	100

* significantly different ($P<0.05$) from saline values (Fisher's exact test or Wilcoxon rank sum test).

Neither flosequinan nor milrinone, at similar hypotensive doses, significantly affected mortality, PVB score or the incidence of VT or VF in survivors. The antiarrhythmic disopyramide eliminated VF and markedly reduced the PVB score and incidence of VT. Isoprenaline, constantly infused to maintain its hypotensive action, caused a significant increase in mortality and increased the incidence of VF though the PVB score was reduced in the few surviving animals. In this model of ischaemically-induced arrhythmias in anaesthetised rats flosequinan has neither proarrhythmic nor antiarrhythmic activity.

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441P EFFECT OF FLOSEQUINAN AND ITS MAJOR METABOLITE, BTS 53 554, ON PHOSPHODIESTERASE (PDE) ISOENZYME TYPES I, II AND III FROM GUINEA-PIG VENTRICLE.

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Flosequinan is currently under clinical trial for the treatment of heart failure. We have previously reported that in a mixed PDE isoenzyme system from bovine heart flosequinan is a non-selective PDE inhibitor of low potency (Frodsham et al 1989). We have now extended our work to investigate the effect of flosequinan and its metabolite BTS 53 554 upon isoenzymes of PDE isolated from guinea-pig left ventricle.

Isoenzymes of PDE were isolated from guinea-pig left ventricle using ion exchange chromatography. Activity was measured by the method of Thompson et al (1979) at 1 μM substrate concentration. Isoenzymes were characterised by their substrate selectivity and regulatory properties. Drugs were dissolved in DMSO to give a final assay DMSO concentration of 2.5%. All drugs were tested for inhibition of types I, II & III PDE activity using either 1 μM cAMP or 1 μM cGMP as substrate. Table 1 shows the IC50 values (mean ± s.e. mean), obtained from three separate isoenzyme preparations (except BTS 53 554 where n=2), and selectivity ratios (SR: type I/type III).

Table 1 Concentration (μM) causing 50% inhibition of cAMP PDE and cGMP PDE

		cAMP PDE				cGMP PDE			
		I	II	III	SR	I	II	III	SR
Flosequinan	(F)	2200 ± 380	2700 ± 180	660 ± 63	3.3	2400 ± 470	600 ± 26	1200 ± 250	2.0
BTS 53 554	(B)	>3000	>3000	1000 ± 180	>3.0	>3000	830 ± 130	2900 ± 150	>1.0
Sulmazole	(S)	3400 ± 830	590 ± 64	470 ± 100	7.2	2800 ± 620	110 ± 8.8	670 ± 70	4.2
Milrinone	(M)	440 ± 130	820 ± 93	5.7 ± 2.0	77	390 ± 140	290 ± 50	390 ± 300	1.0
Zaprinast	(Z)	12 ± 3.0	240 ± 32	2300 ± 720	.005	13 ± 1.3	66 ± 3.8	160 ± 38	.08
IBMX	(X)	22 ± 3.0	62 ± 12	14 ± 1.3	1.6	21 ± 3.4	18 ± 0.6	15 ± 1.7	1.4

The SR's of M and Z were indicative of their type III and type I selective PDE inhibition respectively. The SR's of F and B were very similar to X a non-selective PDE inhibitor and also similar to S (type II/III inhibitor). Overall F and B were the least potent of the compounds tested. This data confirms our previous work suggesting that F and B are quite clearly different to M and Z and similar to X.

We conclude that flosequinan and BTS 53 554 are non-selective inhibitors of type I, II & III isoenzymes from guinea-pig ventricle. Their low relative potencies as inhibitors of guinea-pig PDE isoenzymes questions the relevance of PDE inhibition to the clinical efficacy of flosequinan in heart failure.

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442P THE POTENCY OF TALUDIPINE (GR53992B) IN THE GUINEA-PIG PAPILLARY MUSCLE IS AUGMENTED IN CONDITIONS WHICH MIMIC ISCHAEMIA

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In the isolated guinea-pig papillary muscle, when the medium is replaced by one of lower pH, of lower O_2 tension and elevated [K^+], the potency of some calcium entry blockers (CEBs) is enhanced (Robertson & Lumley, 1989). Thus, verapamil is potentiated 9-fold, diltiazem about 2-fold and nifedipine is not potentiated at all. We have extended the study with the 'ischaemic' papillary muscle assay to the novel dihydropyridine Taludipine (Micheli, *et al.*, 1990), which shares some of the properties (an ionizable basic group and some degree of frequency dependency) that are associated with verapamil.

Papillary muscles, dissected from the right ventricle of male guinea pigs (~450g) were suspended in Krebs buffer (pH 7.4, 32°C) and bubbled with 95% O_2/5% CO_2 under a resting tension of 0.5g and were stimulated at 3Hz at 10V. Ischaemia was imitated by reducing the pH to 6.9, elevating the [K^+] from 5.9 to 9.2 mM and by changing the gas mixture to 55% O_2/40% N_2/5% CO_2 (Robertson & Lumley 1989). Propranolol ($3x10^{-7}$M) was present throughout the experiments. CEBs were pre-equilibrated for 2h and their activity was defined as the negative logarithm of the concentration which displaced a cumulative calcium concentration-effect (E/[A]) curve two-fold ($p(CA_2)$). This was estimated from fitting an equation of the general form: $\log_{10}(\text{dose-ratio} - 1) = r \cdot \log_{10}[CEB] - \log_{10}(CA_2)$.

Table 1 : $p(CA_2)$ values with 95% Confidence Limits from 9 to 12 preparations per estimate.

CEB	$p(CA_2)$ 'Ischaemia'		$p(CA_2)$ 'Normal'		Difference
Verapamil	7.38	(7.2, 8.5)	6.38	(6.2, 7.1)	1.00*
Nifedipine	8.43	(7.9, 9.5)	8.26	(7.4, 9.4)	0.17
(±)-Taludipine	7.36	(7.0, 8.2)	5.87	(5.5, 6.0)	1.49*
(-)-Taludipine	7.45	(6.9, 8.3)	6.25	(5.8, 6.6)	1.20*
(+)-Taludipine	4.78	(4.5, 5.6)	4.62	(4.2, 5.3)	0.16

* - $P<0.05$ $r = 0.43 \pm 0.03$ (s.e.) in ischaemia $r = 0.48 \pm 0.04$ (s.e) in normal

The CEB activity of Taludipine is significantly potentiated in 'ischaemia' (31-fold)(Table 1). This property is only associated with the more active (-)-stereoisomer (16-fold), whilst the weakly active (+)-stereoisomer is not significantly affected (1.5-fold). Thus, this study shows that potentiated activity in 'ischaemia' is not confined to only one class of CEB and that for Taludipine the potentiation is stereospecific.

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443P CHARACTERISATION OF HUMAN PLATELET CYCLIC NUCLEOTIDE PHOSPHODIESTERASE (PDE) ISO-ENZYMES AND THEIR SENSITIVITY TO A VARIETY OF SELECTIVE INHIBITORS

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The aim of the present study was to characterise platelet cytosolic and particulate PDE isoenzymes and to examine the selectivity of isoenzyme inhibitors. Fresh human platelets ($5x10^{11}$/ml)were homogenised in 5 ml of buffer A (20 mM Bis Tris, 2 mM EDTA, 2 mM benzamidine, 20 mM NaCl, 1 mM dithiothreitol, 100 µM PMSF, pH 6.5) and centrifuged (25,000 g x 25 min). The pellet was rehomogenised (5 ml buffer A) and the extract centrifuged (25,000 g x 25 min). The supernatants from the two spins were combined and applied to a Mono-Q ion exchange column (pre-equilibrated in buffer A) attached to a fast protein liquid chromatography system. Bound material was eluted with a NaCl gradient. The pellet from the second spin was used as source of particulate PDE. PDE was assayed using 1 µM substrate as described by Shahid and Rodger (1989).

Four soluble PDE activities were observed: cyclic GMP specific (cG-spec) PDE, cyclic GMP-stimulated (cG-stim; cyclic AMP hydrolysis increased by cyclic GMP) PDE and two other PDE types, eluting at different [NaCl] but both of which showed high affinity for cyclic AMP (cG-inhib PDE; cyclic AMP hydrolysis inhibited by cyclic GMP). The particulate PDE showed 4 fold higher activity for cyclic GMP than cyclic AMP. The latter activity was inhibited by cyclic GMP. The IC50 (µM) values for the effects of isoenzyme inhibitors are shown in Table 1. Milrinone showed weak selectivity for inhibiting cG-inhib PDE, it also inhibited cG-spec PDE. Zaprinast and dipyridamole produced selective inhibition of cG-spec PDE. The new cardiotonic agent Org 30029 inhibited both cG-spec and cG-inhib PDEs. Rolipram was relatively inactive against all PDEs.

Table 1

	cG-spec PDE	cG-stim PDE		cG-inhib PDEa	cG-inhib PDEb	Particulate PDE	
	cGMP	cAMP	cGMP	cAMP	cAMP	cGMP	cAMP
Milrinone	2.8	>250	>250	0.68	0.51	30	0.49
Zaprinast	0.73	110	98	18	20	1.2	13
Dipyridamole	1.6	21	19	73	71	7.8	73
Org 30029	40	>250	>250	16	17	>250	48
IBMX	4.8	29	35	19	20	9.1	13
Rolipram	>250	>250	>250	>250	>250	>250	>250

In conclusion, human platelets contain cG-spec, cG-stim and cG-inhib PDEs in the soluble fraction whilst particulate material shows a mixture of cG-spec and cG-inhib types of PDE activities. Ca/calmodulin stimulated and cyclic GMP-insensitive high affinity cyclic AMP PDEs were not detected. The inhibitory effects of milrinone and Org 30029 on cG-spec PDE, in addition to cG-inhib PDE, may also contribute to the ability of these compounds to inhibit platelet aggregation.

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444P PLATELET SENSITIVITY TO ADRENOCEPTOR STIMULATION IN SHORT- AND LONG-TERM DIABETIC RATS

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Platelets from patients with diabetes mellitus show increased *in vitro* aggregability to a variety of agents (Kwann *et al.*, 1972). We have previously shown in the rat that cardiac responsiveness to adrenergic agents may be altered in diabetes, although these effects depend on the duration of diabetes (Austin *et al.*, 1989). Since α-adrenoceptor stimulation enhances and β-adrenoceptor stimulation inhibits aggregatory responses to other agents, we have examined the effects of diabetes on platelet adrenoceptor responses.

Female Wistar rats (200g) were made diabetic by a single inter-peritoneal injection of streptozotocin (50 mg kg^{-1}). Blood was collected into 3.8% sodium citrate 2 or 12 weeks after injection and platelet rich (PRP) and platelet poor plasma (PPP) prepared. The aggregatory response of PRP was measured using a Payton dual channel aggregometer.

Table 1: Effects of isoprenaline and adrenaline on the responses to 1 µM ADP in platelets from control and diabetic rats

	2 week control:	2 week diabetic:	3 month control:	3 month diabetic:
control ADP	100	100	100	100
+ 100 µM isoprenaline	64.1 ± 11.1*	51.8 ± 11.3*	42.9 ± 9.6**	70.3 ± 17.4+
+ 10 µM isoprenaline	68.9 ± 6.9*	65.2 ± 9.0*	50.6 ± 7.5*	64.7 ± 11.4
Control ADP	100	100	100	100
+ 100 µM adrenaline	192.1 ± 37.7*	251.6 ± 55.4*	195.6 ± 16.8*	179.7 ± 27.5*
+ 10 µM adrenaline	172.3 ± 21.1*	253.4 ± 51.9*	191.4 ± 16.1*	189.2 ± 33.6*

* P < 0.05, ** P 0.005 relative to control ADP response

The aggregation of platelets from control and diabetic rats to ADP (1 µM-10mM) was measured and expressed as a percentage of the maximum deflection to PPP. Aggregation to ADP was unchanged in blood from 2 week diabetic animals but aggregation was significantly increased with platelets from 3 month diabetic animals. Aggregatory responses to ADP were increased from 44.0 ± 4.2 to 60.2 ± 4.0% for 1mM ADP; from 38.0 ± 3.8 to 67.3 ± 3.4% for 100 µM ADP and from 29.0 ± 3.5 to 49.3 ± 4.7% for 3 µM ADP (P < 0.05 for all concentrations).

The effect of adrenoceptor stimulation on platelet responses to a low dose (1 µM) of ADP was also studied. Responses were expressed as a percentage of the response in the absence of the adrenergic agonist (table 1). In the presence of phentolamine (10 µM) and isobutyl methylxanthine (10 µM), isoprenaline attenuated responses to 1 µM ADP to a similar extent in 2 week and 3 month control and diabetic platelets. Adrenaline in the presence of 1 µM propranolol potentiated responses to ADP in blood from controls and diabetics to a similar extent.

These results confirm that diabetes increases aggregatory responses to ADP of platelets from 3 month but not 2 week diabetic rats. Proaggregatory responses to α-adrenoceptor stimulation and the antiaggregatory responses to β-adrenoceptor stimulation, however, do not appear to be affected by the diabetic state.

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445P THE IMMUNOSTIMULATORY ACTIONS OF TUMOUR NECROSIS FACTOR ARE SUPPRESSED BY DEXAMETHASONE

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Tumour necrosis factor-α (TNF-α/ cachectin), is a protein synthesised and secreted by mononuclear phagocytes (Mø) in response to stimulation with bacterial endotoxin, viruses and certain other agents. It is a cytotoxin which causes the necrosis of some tumours *in vivo*, is cytostatic for transformed cell lines *in vitro* and appears to have wide ranging inflammatory effects (Beutler & Cerami 1987). Dinarello *et al*, (1986) have shown that TNF-α is also pyrogenic and it has been suggested that endogenous TNF-α plays a role in mediating the febrile response to bacterial endotoxin as a monoclonal antibody to rabbit TNF-α, injected intravenously, into endotoxin-treated rabbits suppresses both peaks of the biphasic fever. It has recently been shown that the synthetic glucocorticoid dexamethasone (DEX) is antipyretic toward lipopolysaccharide (LPS) and native interleukin 1/endogenous pyrogen, (Abul *et al*, 1987). Many biological actions of DEX are thought to occur by suppressing eicosanoid biosynthesis and this may be involved in the antipyretic action of DEX. Since it is not known whether the antipyretic action of DEX involves attenuation of the actions of TNF-α. We have presently studied the effects of DEX on the pyrogenicity of TNF-α, plasma levels of PGE_2 during TNF-α fever and the release of PGE_2 from Mø incubated with TNF-α.

Male Dutch rabbits (2.0-2.3 kg) were lightly restrained in stocks and their rectal temperatures constantly monitored using thermistor probes. DEX (3 mg/kg) or saline was given i.v. followed by TNF-α (11 μg/kg) 1 hour later. Blood samples were taken from the marginal ear vein prior to any agents and at various intervals after administration of TNF-α. Each blood sample (1 ml) was centrifuged and the plasma acidified (pH 4) and passed through Sep-Pak C_{18} columns. PGE_2 in the methanol eluate was estimated by radioimmunoassay. Mø were prepared from rabbit blood by ficoll density-centrifugation followed by incubation in plastic tissue culture petri-dishes for 2 hours (37 °C, 5 % CO_2, 100 % humidity) after which the adherent cells (Mø) were scraped from the culture dishes. Mø (5×10^5 cells/well of 24-well culture plates) were incubated for 6 hours with 0.5 μg/ml TNF-α and 10 μg/ml DEX after which PGE_2 was measured from in supernatants.

TNF-α produced a biphasic fever with onset after 15 minutes, a first peak which occurred after 45 minutes and a second peak after 3 hours. DEX attenuated both peaks of the febrile response to TNF-α and decreased the magnitude (TRI_4) of the fever from 2.61 ± 0.33 to 1.14 ± 0.32, $P < 0.01$, (means of n = 4 ± s.e.m.). The plasma level of PGE_2 increased in a biphasic manner after TNF-α administration simultaneously with the changes in body temperature. The plasma level of PGE_2 increased from 46.1 ± 22.3 pg/ml, prior to any treatment, to 430.3 ± 92.1 pg/ml 50 minutes after administration of TNF-α and 399.6 ± 69.5 after 3 hours. DEX pre-treatment reduced these levels to 189.4 ± 48.3 pg/ml after 50 minutes and 156.4 ± 33.2 pg/ml after 3 hours (both $P < 0.01$, values are means of n = 4 ± s.e.m.). PGE_2 release from Mø incubated with TNF-α increased from 109.9 ± 10.5 pg/well to 191.6 ± 11.4 pg/well, this was decreased in the presence of DEX to 146.7 ± 12.3 ($P < 0.01$, n = 3 ± s.e.m.). The release of PGE_2 from Mø in the presence of DEX alone was not significantly different than the control incubations i.e. media alone (104.4 ± 7.3 pg/well).

DEX can suppress the immunostimulatory actions of TNF-α both *in vivo* and *in vitro*. The pyrogenic action of TNF-α can be attenuated by DEX possibly by reducing the biosynthesis of PGE_2 as DEX reduced the plasma level of PGE_2 during TNF-α fever and also reduced the release of PGE_2 from TNF-stimulated Mø *in vitro*.

Recombinant TNF and DEX were kind gifts from Dr. L.S. Lin, Cetus Corporation, California and Merck Sharpe and Dohme, Herts, U.K. respectively.

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446P INHIBITION OF HUMAN EOSINOPHIL SUPEROXIDE ANION PRODUCTION BY DEXAMETHASONE

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Elevated numbers of eosinophils are found in the airways and peripheral blood of asthmatic patients and release of eosinophil products have been implicated in the pathophysiology of asthma. Corticosteroids are effective in the treatment of asthma and may inhibit eosinophil function..

We examined the effect *in vitro* of dexamethasone on receptor-stimulated superoxide radical (0_2^-) production by opsonized zymosan in human eosinophils. Eosinophils were purified from peripheral blood, collected into acid citrate dextrose, from 6 asthmatic patients none of whom were receiving oral steroid treatment. White blood cells (WBC) were separated after sedimenting the blood with dextran for 45 min. The WBC fraction was incubated with 10 nM f-methyl-leucine-phenylalanine at 37 C for 10 min to activate the neutrophils and decrease their density (Koenderman et al,1988). The WBC were layered onto continuous density Percoll gradients and distributed throughout the gradients by centrifugation at 1,600 x g for 30min. Sequential fractions were collected, differential counts were performed to identify pure eosinophil populations (>90%), which were pooled and contaminating erythrocytes removed by distilled water lysis. The eosinophils were washed, resuspended in tissue culture medium supplemented with 10% foetal calf serum and incubated for 20 h with various doses of dexamethasone in the presence or absence of the steroid receptor antagonist RU 38486. Viability of cells after culture was assessed by trypan blue exclusion and was >85%.

Eosinophils were stimulated using 1mg/ ml opsonized zymosan, incubated at 37 C for 15 min. 0_2^- was measured by the superoxide dismutase inhibitable reduction of cytochrome C measured at 550nm. Mean control response was 4.468 $\pm$ 0.77 nM $.0_2^-$ per 10^6 cells.

Dexamethasone inhibited the 0_2^- response in a dose-related manner (0.01uM 21.1 $\pm$ 13.9%, n=4; 0.1uM 41.9 $\pm$ 7.9% n=6; 1uM 48.2 $\pm$ 13.9%, n=6). RU 38486 1uM completely prevented inhibition observed with 1 uM dexamethasone . Baseline 0_2^- release was low and not affected by incubation with dexamethasone.
We conclude that in vitro treatment of human eosinophils with dexamethasone is able to inhibit $.0_2^-$ generation through a mechanism, which involves steroid receptor occupancy.

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447P THE EFFECT OF THE LIPOXYGENASE INHIBITOR BW A797C ON EICOSANOID FORMATION FOLLOWING PYROGEN ADMINISTRATION

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Circulating levels of prostaglandin E_2 increase following the administration of various pyrogens including the interferon-inducer polyinosinic:polycytidylic acid (Poly I:C). The non-steroidal anti-inflammatory cyclo-oxygenase inhibitor ketoprofen inhibits both the pyrogen-induced fever and the elevation in blood PGE_2 level, (Rotondo *et al*, 1988). Little information is available regarding the role of other eicosanoids such as the leukotrienes during fever, either with respect to whether the circulating level of these agents change or whether lipoxygenase inhibitors have any effect on pyrogen-induced fever. In the present study we have investigated the effects of a novel lipoxygenase inhibitor, BW A797C, on body temperature, plasma levels of PGE_2 and the *ex vivo* synthesis of (leukotriene B_4) LTB_4 during Poly I:C-induced fever.

Male Dutch rabbits (2.0-2.3 kg) were lightly restrained in stocks and their rectal temperatures constantly monitored using thermistor probes. A blood sample (≈ 3 ml) was taken from the marginal ear vein, 30 minutes later either saline or Poly I:C (5 μg/kg i.v.) was administered and 10 minutes later the animal was given either glycofurol vehicle or BW A797C (5 mg/kg i.v.). A second blood sample was taken 90 minutes after Poly I:C administration coinciding with the first peak of the Poly I:C fever and a third blood sample was taken after 3 hours coinciding with the second peak of the biphasic fever. A portion of each blood sample (1 ml) was centrifuged and the plasma acidified (pH 4) and passed through Sep-Pak C_{18} columns. PGE_2 in the methanol eluate was estimated by radioimmunoassay. Two 0.5 ml aliquots of each blood sample were incubated for 30 minutes with calcium ionophore A23187 (10 μl of 0.75 mg/ml in DMSO) after which the tubes were centrifuged and the plasma level of LTB_4 was estimated as described previously (Tateson *et al*, 1988).

Poly I:C + glycofurol produced a biphasic increase in body temperature with a first peak at 90 minutes $\Delta T = 0.75 \pm 0.15$ °C and a second peak around 3 hours after injection of Poly I:C, $\Delta T = 0.99 \pm 0.10$ °C (all results are expressed as means of $n = 4 \pm$ s.e.m.). An almost identical response was observed if Poly I:C and BW A797C were both administered, $\Delta T = 1.04 \pm 0.09$ °C 90 minutes after injection and $\Delta T = 1.18 \pm 0.08$ °C after 3 hours. Glycofurol alone did not significantly affect body temperature. The plasma level of PGE_2 increased approximately 5-fold from 35.0 ± 5.5 pg/ml before any treatment to 171.3 ± 40.8 and 157.0 ± 33.0 at 90 minutes and 3 hours after Poly I:C + glycofurol respectively. The elevated level of PGE_2 with Poly I:C + glycofurol treatment was not significantly different from that observed with Poly I:C + BW A797C (200.8 ± 56.9 pg/ml after 90 minutes, and 158.1 ± 34.6 after 3 hours). In contrast the *ex vivo* production of LTB_4 was much less in blood from animals which had been given Poly I:C + BW A797C (3.8 ± 1.6 ng/ml after 90 minutes, $P < 0.01$, and 32.3 ± 10.6 ng/ml after 3 hours) than from animals given Poly I:C + glycofurol (34.4 ± 4.4 ng/ml and 46.5 ± 6.1 ng/ml after 90 minutes and 3 hours respectively). The LTB_4 recovered in blood taken from animals prior to any treatment was 36.1 ± 3.9 ng/ml.

These data suggest that BW A797C specifically inhibits the production of LTB_4 by inhibiting lipoxygenase, but not the prostanoid PGE_2. As BW A797C did not reduce either the Poly I:C-induced fever or elevated PGE_2 level this would suggest that LTB_4 is not involved in initiating the febrile response to Poly I:C.

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448P THE INFLUENCE OF EXTRACELLULAR CALCIUM AND DIFFERENT LOADING CONCENTRATIONS OF FURA-2/AM ON THE CALCIUM RESPONSE TO PAF IN GUINEA-PIG EOSINOPHILS

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The primary disadvantage of the use of calcium-sensitive fluorescent indicators is the risk of intracellular buffering which prevents the accurate assessment of agonist-induced changes. Herein we report that in guinea-pig eosinophils the characteristics of the calcium response to PAF, namely the sensitivity, the peak height and dependence on intracellular and extracellular calcium are modified by relatively low fura-2/AM loading concentrations.

We undertook a comparison of the PAF response in guinea-pig eosinophils loaded in the presence of either 2.5μM or 0.5μM fura-2/AM. Using these conditions calcium transients were quantified and their dependence on intra and extracellular calcium determined.

Male guinea-pigs sensitised by 2 injections of horse serum i.p. were subject to peritoneal lavage using calcium and magnesium-free Hanks Buffered Salts Solution and the resulting cells separated on a metrizamide gradient, to give a viable purified pool of eosinophils. Incubations with fura-2/AM were performed in RPMI 1640 medium supplemented with 0.1% BSA at room temperature for 30 minutes at a density of 4 million cells per ml. Immediately before assay the cells were washed with hepes-buffered Tyrode solution, containing 1mM calcium and resuspended in 1.5ml of the same. Fluorescence was measured using a Perkin-Elmer LS-5B luminescence spectrometer at excitation wavelengths 340 and 380nm and emission at 509nm. The results presented are the mean of at least three separate experiments in each case.

The results indicate that there is an inverse relationship between loading concentrations of fura-2/AM in the range of 0.5-2.5μM and the agonist induced peak height. That is, peak height fell from a maximum of 870nM to 500nM with increasing concentrations of fura-2/AM. Furthermore,the sensitivity of the calcium response to PAF was reduced with increasing loading concentrations. That is, the lowest concentration of PAF required to produce a rise in calcium increased from 100pM when cells were loaded with 0.5μM fura-2/AM to 1nM when loaded with 2.5μM dye. Experiments involving the use of calcium-free buffer and a minimal concentration of EGTA implied that the dependence of the calcium transient on extra-cellular calcium was increased with increasing loading concentrations of the dye. The proportion of the initial calcium peak height arising from extracellular calcium fell from 48% at 2.5μM fura-2/AM to 30% at 0.5μM dye in response to 1μM PAF. The maintained phase of the calcium response to PAF was abolished in the absence of extracellular calcium. Thus the characteristics of the calcium transient induced by PAF in eosinophils were greatly dependent on the fura-2/AM loading concentrations and extracellular calcium. Therefore the optimum concentration of fura-2/AM should be determined for each individual cell type.

449P EFFECTS OF SULPHASALAZINE AND BALSALAZIDE ON CELL MEMBRANE STABILITY AND HISTAMINE RELEASE

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Sulphasalazine (SASP) and balsalazide (BSZ) are potent drugs for ulcerative colitis therapy. They also reduce ethanol-induced gastric damage in rats, suggesting a potential for peptic ulcer treatment (Cho *et al.*,1987; Parke *et al.*,1986). Our aim was to see if they act via 'cell membrane stabilization'.
We studied their effect on: 1. histamine release from (a) mite allergen-stimulated human basophils (HB), isolated from heparinized blood samples of allergic subjects by methylcellulose sedimentation of red cells, and incubated with 2μg ml^{-1} allergen in HEPES-buffered tyrode solution (pH 7.4 at 37°C) with and without test agents, (b) compound 48/80(0.3μg ml^{-1})-stimulated rat peritoneal mast cells (RPMC,male Wistar rats). Histamine was assayed fluorometrically. 2. melittin (7.5μg ml^{-1})-induced lysis of human erythrocytes (Mizushima *et al.*,1970). SASP and BSZ were compared with related compounds: 5-aminosalicylic acid (5-ASA, active moiety common to SASP and BSZ), sulphapyridine (SP, carrier moiety of SASP), 4-aminobenzoyl-β-alanine (4-ABA, inert carrier moiety of BSZ), and disodium cromoglycate (DSCG). DSCG, SASP, BSZ and 5-ASA but not 4-ABA or SP produced significant inhibition of 48/80- induced histamine release from RPMC (Table 1). By contrast only low dose SASP (2.5x10^{-5}M) and high dose SP (6.3x10^{-4}M) caused some inhibition of histamine release from HB (9% and 15% respectively). SASP and BSZ (2x10^{-5} to 5x10^{-4}M) reduced melittin-induced haemolysis dose-dependently (25% with 5x10^{-4}M,p<0.001).

Table 1 Inhibition of histamine release from rat peritoneal mast cells (%, mean ± SD, n=3)

	DSCG	SASP	BSZ	5-ASA	4-ABA	SP
2x10^{-5}M	14.0±3.8	11.5±0.7	25.1±1.6^{a}	14.8±2.6	-2.1±1.0	2.7±3.1
1x10^{-4}M	46.1±2.4^{c}	73.4±4.0^{c}	42.1±3.3^{b}	29.5±7.8^{a}	4.3±7.1	2.4±6.9

Student's t-test (on original values) $^{a}p<0.05$; $^{b}p<0.01$; $^{c}p<0.001$, vs 48/80-treated controls.
Our results are compatible with those of Barrett *et al.* (1985) who showed that SASP inhibited IgE-mediated histamine release from RPMC and cultured mouse mast cells but not HB. The inhibition by SASP, BSZ and 5-ASA of histamine release and red cell lysis suggests that they have cell membrane stabilizing effects.

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450P THE ROLE OF CALCIUM IN THE RELEASE FROM ENDOTHELIAL CELLS OF EDRF AND PROSTACYCLIN INDUCED BY DIFFERENT AGONISTS

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Receptor mediated release from endothelial cells of endothelium-derived relaxing factor (EDRF) and prostacyclin (PGI_2) are coupled (de Nucci *et al.*, 1988a) and are differentially controlled by extra- and intracellular Ca^{2+} (Lückhoff, 1988). The present study was designed to elucidate the relevance of Ca^{2+} fluxes for EDRF and PGI_2 release stimulated by BK (receptor mediated), an alkaline buffer (D-arginine free base, non-receptor mediated; de Nucci *et al.*, 1988b) or poly-L-arginine or poly-L-lysine (basic polyamino acid). The intracellular Ca^{2+} antagonist TMB-8 (Malagody & Chiou, 1974) or Ca^{2+} free Krebs' solution (including EGTA, 0.2 mM) were used to remove the available intra and extracellular Ca^{2+}, respectively. We also examined the effect of TMB-8 on PGI_2 formation induced by arachidonic acid (AA), the endogenous source of PGI_2.
Bovine aortic endothelial cells were cultured on microcarrier beads and packed into minicolumns (Kondo *et al.*, 1989). EDRF release was detected in the effluent from the minicolumns by activation of isolated soluble guanylate cyclase and subsequent cGMP formation determined by specific radioimmunoassay (Kondo *et al.*, 1989). PGI_2 release was monitored by radioimmunoassay of 6-keto-$PGF_{1\alpha}$.
EDRF release induced by BK (10 pmol) was significantly reduced by either TMB-8 (0.1 mM; n=4) or Ca^{2+} free buffer (n=4; $p<0.05$). The release of EDRF induced by poly-L-arginine (100 pmol) or poly-L-lysine (250 pmol) was unaffected by TMB-8 (n=4) but significantly inhibited, although not abolished in Ca^{2+} free buffer (n=4). D-arginine free base (2 µmol) induced a release of EDRF which was unaffected by TMB-8 but abolished by a Ca^{2+} free medium (n=4). BK (10 pmol), poly-D-lysine (100 pmol) or poly-L arginine (50 pmol) but not D-arginine free base (2 µmol) induced the release of PGI_2. In each instance this release was unaffected by a Ca^{2+} free medium and abolished by TMB-8 (n=4). AA (0.3 and 6 nmol) induced the release of PGI_2 which was significantly inhibited by TMB-8 (n=3-4). This finding indicates that either cellular synthesis of PGI_2 requires intracellular Ca^{2+} mobilisation or TMB-8 has some inhibitory effect on cycloxygenase or prostacyclin synthase.
It is clear that the release of EDRF induced by D-arginine free base has an absolute requirement for extracellular Ca^{2+} but that BK and, to a lesser extent, basic polyamino acids can mobilise intracellular Ca^{2+}, suggesting that the release of EDRF can be differentially controlled by intra and extracellular Ca^{2+} depending on the type of agonist used.

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451P EDRF MIMETIC IMPROVES REPERFUSION INJURY BUT NOT IN THE PRESENCE OF SUPEROXIDE AND HYDROXYL FREE RADICAL SCAVENGERS - THE SECOND EDRF?

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During hypoxia production of the vasodilator EDRF-NO is enhanced (Pohl and Busse 1989) but endothelin may be elaborated (De Nucci et al 1988). Superoxide radicals, generated during reperfusion, annihilate EDRF-NO, but release a second EDRF (Hong et al 1989). Sodium nitroprusside elevates smooth muscle cGMP, bypassing EDRF-NO generation, and counteracts endothelin. Thus we investigated sodium nitroprusside in an animal model of lower limb ischaemia and reperfusion injury in the presence and absence of superoxide and hydroxyl radical scavengers.

A tourniquet was applied to one hind limb of male Sprague Dawley rats for 6 hours. 30 mins before revascularisation 29 animals began an infusion of sodium nitroprusside (SN) 1.5 μg/kg/min. 8 of these animals received superoxide dismutase 35,000 iu/kg/h plus catalase 4000,000 iu/kg/h(SN+SD+C), whilst a further 6 received dimethylthiourea 500 mg/kg/h(SN+DMTU). 12 animals acted as controls. Gastrocnemius muscle blood flow was assessed 10, 120 and 240 min after reperfusion by injection of 15 μm radiolabelled microspheres (^{46}Sc, ^{85}St, ^{141}Ce) and a perfusion index calculated as the ratio between the study and the contralateral limb (Table 1).

Table 1. The perfusion ratios in control and treated rats. Perfusion Index (median & i.q. range).

	N	Time (min) 10		120		240	
Control	12	0.12	(0.02-0.43)	0.48▪▪°°	(0.11-0.70)	0.05*▪	(0.01-0.38)
SN	15	0.21▪▪	(0.15-0.42)	0.59**++	(0.32-1.15)	0.41*▪▪++	(0.11-1.33)
SN+SD+C	8	0.02▪▪	(0.00-0.03)	0.00**°°	(0.00-0.01)	0.01▪▪	(0.00-0.03)
SN+DMTU	6	0.08	(0.00-0.15)	0.01++▪▪	(0.00-0.02)	0.00++▪	(0.00-0.01)

The significance of differences between any two values (identified using the same symbols) within each time period is indicated as * or ▪ $p<0.05$, **, ▪▪, ++ or °° $p<0.01$ (Mann Whitney U test).

This data suggests that the EDRF mimetic sodium nitroprusside significantly improves reperfusion injury probably by overcoming the effect of endogenous endothelin-1. In the presence of superoxide and hydroxyl free radical scavengers this effect is lost suggesting a free radical mediated intermediate in the vasodilation - perhaps the second EDRF?

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452P AGONIST- AND TISSUE-DEPENDENCE OF SUSCEPTIBILITY OF ENDOTHELIUM-DEPENDENT RELAXATIONS TO L-NAME

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Analogues of l-arginine, e.g. N^G-nitro-L-arginine methyl ester(L-NAME), inhibit endothelium-dependent vascular relaxation, presumably by blocking the synthesis of nitric oxide(Rees *et al*, 1990). This study shows that inhibition by L-NAME exhibits tissue- and agonist-dependence and suggests a possible explanation for these phenomena. Changes in isometric force were recorded from rings of rat aorta(RA) or rabbit jugular vein(RbJV) in which endothelium-dependent relaxations were elicited either with acetylcholine(ACh) or the endothelial 5-HT receptor agonist (±)alpha-methyl-5-HT(AMT).

ACh caused concentration-related relaxations of RbJV($p[A_{50}]=8.38\pm0.16$,n=5) and RA ($p[A_{50}]=7.27\pm0.08$,n=5). In RA ACh relaxations were virtually abolished by 10uM L-NAME. In RbJV 0.3-30uM L-NAME inhibited relaxations to AMT but was ineffective against ACh,although 100uM caused a 20% decrease in asymptote and a 0.4 log unit right-shift of the curve. There was no evidence for tissue differences in muscarinic receptor type since affinity estimates for the M_3 receptor antagonist 4-diphenylacetoxy-N-methylpiperidine methobromide (4-DAMP:$pK_B=9.01\pm0.12$,17d.f.,RA;9.24 ± 0.16,29d.f.,RbJV) and for ACh itself ($pK_A=6.08\pm0.22$, 10d.f.,RA;6.09 ± 0.09,10d.f.,RbJV) were similar in each case. ACh relaxations were unaffected by 1uM glibenclamide, suggesting that a K^+ channel-linked hyperpolarising factor was not involved, and both tissues also relaxed completely to glyceryl trinitrate(1nM-1uM). The inhibitory effect of 10uM L-NAME was restored when the efficacy of ACh in RbJV was reduced by inactivating a fraction of the muscarinic receptors so that ACh now behaved as a partial agonist, in this respect resembling the behaviour of AMT in this tissue(Leff *et al*, 1987).These results are consistent with the predictions of a theoretical model analogous to that described by Black *et al*(1980). Simulations based on this model illustrate that inhibition by L-NAME will be reduced under conditions of highly efficient receptor-effector coupling. Consequently, resistance to blockade by analogues of L-arginine is not necessarily evidence for the lack of involvement of nitric oxide in endothelium-dependent relaxant responses.

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453P ENDOTHELIUM-DEPENDENT CONTRACTION TO 5-HT IN RABBIT ISOLATED CEREBRAL ARTERIES

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Endothelium-dependent relaxation can be stimulated by 5-hydroxytryptamine (5-HT) in a number of blood vessels from the peripheral vasculature (Cocks & Angus, 1983; Leff et al, 1987). This action of 5-HT appears to be mediated by 5-HT_1 receptors, as it can be blocked with methiothepin but not with ketanserin (Leff et al, 1987). Cerebral arteries appear to respond in a different way to 5-HT. Conner & Feniuk (1989) were unable to demonstrate endothelium-dependent relaxation to 5-HT in canine cerebral arteries, suggesting that the ability of the endothelium to attenuate contraction to 5-HT might be explained by the spontaneous release of EDRF. We have investigated responses to 5-HT in rabbit cerebral arteries and find that 5-HT can produce endothelium-dependent contraction in partially contracted arteries, an effect which appears to be mediated by 5-HT_1 receptors.

Isolated segments of basilar artery were submaximally contracted with histamine (1μM; 1.5 ± 0.2g, n=6) and cumulative concentration-response curves constructed with either 5-HT, 5-carboxamidotryptamine (5-CT) or 8-OH DPAT. The responses were compared with segments whose endothelium had been removed by gentle rubbing, and the success of this manoeuvre assessed by failure to produce relaxation in response to acetylcholine. Both 5-HT and 5-CT produced additional smooth muscle contraction in the basilar artery, but only if the endothelium was intact. The threshold contraction was produced with either 1nM 5-HT or 5-CT, and was followed by concentration-dependent increments in tension to a maximum response with 1μM in both cases. The maximum contraction produced in response to 5-HT represented an additional 22% of the original histamine contraction (n=6), and with 5-CT an additional 13% (n=11). These increases were not seen in the absence of endothelial cells. 8-OH DPAT failed to produce any further contraction in the presence of histamine, whether or not the endothelium was intact. However, the spontaneous fade of contraction was slower in artery segments exposed to 8-OH DPAT than in parallel, time-control segments contracted with histamine. This effect was independent of the endothelium and suggested a direct contractile action of 8-OH DPAT on the smooth muscle cells.

These data indicate that in rabbit cerebral arteries the endothelium can produce smooth muscle contraction when stimulated by 5-HT. As 5-CT had a similar action to 5-HT, the receptor mediating this effect may be a 5-HT_1 receptor. The failure of 8-OH DPAT to produce endothelium-dependent contraction suggests that if this is the case, then the it is not a 5-HT_{1A} receptor.

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454P EFFECT OF U74006F ON OEDEMA AND INFARCT VOLUMES FOLLOWING PERMANENT OCCLUSION OF THE MIDDLE CEREBRAL ARTERY IN THE RAT

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Oxygen radicals and lipid peroxidation have both been implicated in the pathophysiology of ischaemic damage (Braughler & Hall, 1989). The lipid peroxidation inhibitor U74006F has been shown to reduce neuronal damage and oedema using post mortem methods of assessment (Hall et al, 1988; Hall et al, 1990; Young et al, 1988). In this study we report effects of U74006F on ischaemia induced cerebral oedema development in vivo using nuclear magnetic resonance (NMR) imaging techniques.

To induce ischaemia, the left middle cerebral artery of male Sprague-Dawley rats (200-250g) was occluded by electrocautery, and the rats were injected with U74006F (3mg/kg i.v. 10min and 3h post occlusion and 10mg/kg i.p. 16h and 24h post occlusion) or vehicle.

Twenty-four hours after surgery the animals were subjected to NMR imaging under sodium pentobarbitone anaesthesia, using a 4.7 tesla Bruker Biospec imaging spectrometer. Oedema was visualised and its volume quantified. Infarct volume was assessed histologically after 3d (haematoxylin and eosin).

The main regions of the brain affected were the cortex and the caudate-putamen of the occluded hemisphere. The oedema volume was 201±34 mm^3 [7] (means.e.mean [n]) for the vehicle treated animals, and 118±21 mm^3 [7] ($P = 0.060$, Student's t-test) in the animals treated with U74006F. The area of oedema was significantly reduced ($P<0.05$, Student's t-test) in the U74006F treated animals at coronal levels 0.0mm, 2.0mm and 10.0mm posterior to bregma. From the histological sections, the infarct volume in the vehicle treated animals was 119±43mm^3 [5], and for the U74006F treated animals the infarct volume was 71±32mm^3 [4] ($P = 0.391$, Student's t-test).

Thus U74006F given post-MCA occlusion may be of benefit in reducing ischaemic sequelae. The mechanism of action may involve reduction of lipid peroxidation as suggested by Hall & Braughler (1986). We cannot, however, rule out other mechanisms of action, such as an effect to stimulate collateral circulation.

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455P HETEROGENEITY OF [^{125}I]-SARAFOTOXIN S6b BINDING SITES IN RAT TISSUES

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Sarafotoxin S6b is a cardiotoxic snake venom whose primary amino-acid sequence is highly homologous with the vasoconstrictor peptide endothelin-1 (ET-1) and it has been reported to bind to the same binding sites as ET-1 in rat atrium and brain (Ambar *et al.*, 1989). We therefore investigated [^{125}I]sarafotoxin S6b binding in rat aorta and cerebellum in order to evaluate this novel ligand and we have compared its binding in the cerebellum with that of [^{125}I]endothelin-1.

Tissues were obtained from male Sprague-Dawley rats (200-300 g; Charles River France) killed by stunning and decapitation. Cerebellum was homogenized in 10 vol. ice-cold buffer (50mM HEPES; 1mM 1,10 phenanthroline; pH 7.4) in a Teflon-in-glass homogenizer with 10 up-and-down strokes. Aortae, cut into 3mm lengths, were homogenized in 7.5 vol. of the same buffer with 2 x 20s bursts in a Polytron at setting 7. Homogenates were centrifuged at 1000g for 10min at 4°C. Protein concentration in the supernatants, which were used for binding experiments, was determined by the Lowry method. Incubations (2h at 25°C), in 0.5ml , used 0.1-0.15mg (cerebellum) or 1mg (aorta) protein in a 50mM HEPES buffer (pH 7.4) containing 1mM 1,10 phenanthroline, 0.1% bovine serum albumen and 140μg/ml bacitracin (all from Sigma Chemical Co.). Bound and free ligand were separated by filtration through Whatman GF/B filters. Saturation experiments used 12 concentrations (7pM-10nM) of [^{125}I]sarafotoxin S6b or [^{125}I]ET-1 (Amersham International) and non-specific binding was defined by 1μM ET-1. Inhibition experiments were identical except that a single concentration (25-50pM) of radioligand and 11 concentrations (1pM-1μM) of ET-1 or sarafotoxin S6b (Peptide Institute, Osaka, Japan) were used.

Specific [^{125}I]sarafotoxin S6b binding was 71-91% of the total in the aorta and 91-98% in the cerebellum. There were no differences in the displacement of this ligand by either 1μM ET-1 or 1μM sarafotoxin S6b. In the cerebellum, [^{125}I]sarafotoxin S6b bound to a single binding site (Hill slope, n_H, = 0.90±0.05; n = 3) with a dissociation constant (K_d) of 0.24±0.06nM; maximum binding capacity was 233±70fmol/mg protein compared to 321±58fmol/mg protein (n = 5) for [^{125}I]ET-1. In 3 aortic homogenates, analysis of [^{125}I]sarafotoxin S6b binding isotherms using LIGAND (Munson & Rodbard, 1980) gave a significantly better fit to a 2-site model. The K_d values were 3.0±1.5nM and 145±12pM with relative abundances of 70±18% and 29±17% respectively. In inhibition studies in the cerebellum the K_i values for sarafotoxin S6b inhibiting the binding of [^{125}I]sarafotoxin S6b and [^{125}I]ET-1 were respectively 0.51±0.07nM (n_H=1.0±0.1; n = 3) and 0.23±0.06nM (n_H=1.1±0.1; n = 6). The corresponding values for ET-1 were 0.64±0.23nM ([^{125}I]sarafotoxin S6b; n_H=0.9±0.1; n = 3) and 0.83±0.15nM ([^{125}I]ET-1; n_H = 1.1±0.1; n = 6).

In conclusion [^{125}I]sarafotoxin S6b is a useful tool for the study of ET-1 binding sites. In the cerebellum it appears to label the same population of sites as [^{125}I]ET-1, has a very low non-specific binding and the interaction between sarafotoxin S6b and ET-1 appears to be competitive. In the aorta there appears to be more than one binding site for this cardiotoxin and the nature of these sites in relation to possible multiple receptors for members of the endothelin/sarafotoxin family of peptides remains to be determined.

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456P INHIBITION OF NORADRENALINE-STIMULATED ^{45}Ca UPTAKE IN RAT AORTA BY CROMAKALIM AND ITS (-) ENANTIOMER, LEMAKALIM (BRL 38227)

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The K channel activator cromakalim (CK) relaxes smooth muscle via mechanisms additional to closure of voltage operated Ca channels (Cook et al., 1988). Chiu et al. (1988) reported that CK inhibited noradrenaline (NA) stimulated ^{45}Ca-uptake by rabbit aorta. This was not, however, demonstrated to be related to K channel opening. In the present study, the effects of CK, and its enantiomers on 45Ca-uptake by rat aorta are compared with effects on 86Rb-efflux.

For Ca-uptake studies, segments of thoracic aorta from male Sprague Dawley rats were incubated for 15 min in HEPES-buffered salt solution, pH 7.4 (HPS), at 37°C containing 1mCi/l ^{45}Ca and appropriate drugs. Tissues then underwent six 15 min washes in ice-cold HPS containing 10mM $LaCl_3$ before being blotted, weighed, and their ^{45}Ca content measured. ^{86}Rb-efflux was measured using the methodology described by Foster (1989).

NA (3μM) stimulated ^{45}Ca-uptake was only partially blocked by verapamil (10μM), whilst KCl (45mM) stimulated 45Ca-uptake was fully blocked at this concentration. However, NA stimulated ^{45}Ca-uptake was completely inhibited by CK (10μM) and its (-)enantiomer lemakalim (1μM), whilst the (+) enantiomer BRL 38226 (10μM) was without effect. CK had no effect on KCl-stimulated ^{45}Ca-uptake.

	Stimulated ^{45}Ca-Uptake (*)	
Treatment	Noradrenaline (3μM)	KCl (45mM)
Control	154±4 (n=63)	175±5 (n=29)
10μM Verapamil	121±5 (n=8)	107±12 (n=6)
10μM Cromakalim	103±6 (n=11)	187±8 (n=8)
1μM Lemakalim	111±9 (n=8)	-

*expressed as a % of basal uptake in non-stimulated aorta ±s.e.m.

Lemakalim (1μM) stimulated ^{86}Rb-efflux from aorta by 145±26% (n=6) and this was blocked by glibenclamide (5μM). Using the same concentrations, glibenclamide also prevented lemakalim inhibiting NA stimulated ^{45}Ca-uptake. With 10μM concentrations of both glibenclamide and lemakalim, however, only a partial blockade of ^{86}Rb-efflux was seen, and lemakalim still inhibited NA stimulated ^{45}Ca-uptake.

In summary, CK can inhibit NA stimulated ^{45}Ca-uptake in rat aorta more effectively than can verapamil. This activity resides in the (-)enantiomer, and appears to be related to the opening of K channels.

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457P EFFECT OF SULFONYLUREAS ON CROMAKALIM-INDUCED RELAXATION OF KCl PRE-CONTRACTED RABBIT, CAT AND RAT ISOLATED CEREBRAL ARTERIES

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The effects of cromakalim (CROM) and its inhibition by the sulfonylurea's glibenclamide (GLIB) and tolbutamide (TOL) on KCl precontracted rabbit (RAB) and cat isolated basilar (BA) or middle cerebral (MCA) arteries and rat BA were investigated.

Ring segments were set up in Krebs Solution for recording of isometric tension. Only vessels with an intact endothelium were used, assessed by application of acetylcholine (10 uM) on histamine (100 uM) (RAB) or 5-hydroxytryptamine (100 uM) (rat and cat) spasm. Concentration-effect (C-E) curves to CROM (10 nM - 500 uM) on elevated tone (20 - 50 mM KCl) were constructed and repeated (on 20 or 30 mM KCl) in the absence or presence of GLIB (0.1 - 1.0 uM) or TOL (10 uM).

KCl produced contraction of all vessels studied. The pD_2 (-logEC_{50}) ± s.e.mean for KCl (E_{max} (mN) ± s.e.mean) were for the RAB: BA 1.53 ± 0.04 (10 ± 1.3), MCA 1.43 ± 0.02 (7.3 ± 1.1); for the cat: BA 1.51 ± 0.06 (9.9 ± 1.0), MCA 1.41 ± 0.04 (13.5 ± 2.8); and for the rat: BA 1.30± 0.05 (7.1 ± 0.6) (n = 7 - 14). CROM produced relaxation of precontracted arteries as shown in table 1.

Table 1

	RAB BA		RAB MCA		CAT BA		CAT MCA		RAT BA	
	pD_2	E_{max}	pD_2	E_{max}	pD_2	E_{max}	pD_2	E_{max}	pD_2	E_{max}
KCl 20mM	7.21±0.05	107± 3	no spasm		6.20±0.04	101± 9	6.26±0.07	99± 6	no spasm	
KCl 30mM	7.11±0.02*	96±11	6.48±0.07*	74± 6	5.92±0.26*	66±14*	5.96±0.27*	81± 5*	no spasm	
KCl 50mM	6.62±0.09*	7.5± 2*	5.21±0.03*	21± 7*	4.95±0.13*	28± 7*	4.25±0.02*	40± 7*	4.70±0.24	12± 5

* $p < 0.05$ from 20 mM (30 mM KCl RAB MCA only) (Duncan's multiple range test). Apparent pD_2 and E_{max} are quoted for 50 mM data. pD_2 = -logEC_{50} ± s.e.mean; E_{max} (% relaxation) ± s.e.mean. n = 4 - 18

CROM was more potent on RAB BA as compared to any other vessel. GLIB or TOL did not effect KCl induced spasm. However, CROM induced relaxations were blocked by GLIB (0.1 - 1.0 uM) whereas TOL (10 uM) had only a small or no effect. Respective pA_2 values ± s.e.mean for GLIB against CROM (Van Rossum, 1963) and slope ± s.e.mean of the Schild regression (Arunlakashana and Schild, 1959) in parenthesis were RAB BA: 6.6 ± 0.09 (1.4 ± 0.21:$p < 0.05$ from 1.0); cat BA 7.1 ± 0.1 (0.8 ± 0.2) and cat MCA 6.5 ± 0.15 (0.8 ± 0.3). A pA_2 value for GLIB against CROM on RAB MCA was not obtained, however 1 uM GLIB produced a similar concentration ratio shift (9.1 ± 3.0; n = 5) as observed on RAB BA (8.1 ± 0.4; n = 6).

This study shows that CROM produces relaxation of cat and RAB isolated cerebral arteries by a similar mechanism identified in other smooth muscle preparations. However, CROM had only a small effect on KCl precontracted rat BA.

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458P LIGUSTRAZINE IS A VASORELAXANT OF PULMONARY ARTERY AND PULMONARY RESISTANCE VESSELS

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The semisynthetic herbal remedy, ligustrazine (LIG) (Tetramethylpyrazine HCl), has been used for many years in China for the treatment of cardiovascular disorders. Recently it has been demonstrated (Cai & Barer, 1989) that LIG attenuates the changes in the pulmonary vasculature which occur during exposure to chronic hypoxia in the rat, but the mechanism of this effect is not understood.

We have investigated the effect of LIG (Pharmacy No 3, Shanghai, PRC) on isolated lobar pulmonary arteries (PA) and pulmonary resistance vessels (PRV) of the rat in vitro. Vessels (n=8) were obtained from female Wistar rats by dissection of bronchovascular bundles and were studied by comparison of pairs mounted in a small vessel myograph in physiological saline solution perfused with 95% O2 5% CO2. A resting tension of 1g was applied and vessels were maximally preconstricted following determination of a dose-response curve to KCl.

LIG caused a dose-dependent relaxation of PA and PRV (Table) which was maximal at 4 minutes in the PA but was slower to reach maximum in the PRV.

	Dose of LIG (mcg/ml)						
	0	2	60	200	600	2000	4000
% Relaxation							
PRV	0	-2	3	9	29	87	102
PA	0	-2	3	40	70	86	86

LIG potently relaxes potassium-preconstricted PA and PRV. However an effect of LIG on pulmonary vascular remodelling in the chronically hypoxic animal rather than a direct vasodilator action may underlie the amelioration of hypoxic change observed by Cai and Barer.

Cai, Y. & Barer, G.R. (1989) Clin Sci 77, 515-520.

459P PULMONARY VASODILATION BY LIGUSTRAZINE

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We studied acute and chronic effects of ligustrazine (LIG, tetramethylpyrazine HCl), principle of an ancient Chinese herbal remedy on pressure/flow relations in the rat lung. Isolated lungs of control (C) and chronically hypoxic rats (CH, 2-3 wks in 10% O2) were perfused with blood of normal pH and haematocrit at 38°C during ventilation with air + 5% CO2 (normoxia) or 2-3% O2 + 5% CO2 balance N2 (hypoxia). Pulmonary artery pressure (P) and flow ($\dot{Q}$) were displayed on an XY recorder, P/$\dot{Q}$ lines were linear except at very low flow and were extrapolated to an intercept on the P axis; so that $Q \propto P\text{-INT}$. The slope of the P/$\dot{Q}$ line equalled vascular resistance; INT was due to alveolar pressure (kept > left atrial pressure) or to muscle tone in small collapsible vessels. In both C and CH rats hypoxia increased both slope and INT. CH rats had pulmonary hypertension and slopes and INTs > C rats, attributable to structural changes in vessels, including reduced vascular compliance and muscularization of normally thin-walled arterioles. The right ventricle (RV) hypertrophied.

LIG, given acutely during hypoxia, reduced both slope and INT in a dose dependent manner in 5 C and 6 CH rats, the largest dose returned the P/$\dot{Q}$ line to normoxic values.

Table 1 Effect of ligustrazine on P/Q lines

	Air	Hypoxia	0.5 mg cumulative dose LIG	1	2	4
C rats (slope	0.6±0.08	1.13±0.23	0.95±0.19	0.84±0.13	0.71±0.12	0.63±0.07
(INT	6.5±2.1	12.5 ±4.1	11.0 ±3.5	9.2 ±2.7	7.4 ±2.8	5.8 ±1.6
CH rats(slope	1.2±0.18	1.39±0.29	1.22±0.18	1.09±0.16	1.00±0.12	0.85±0.09
(INT	17.1±4.6	28.9 ±7.1	23.3 ±5.6	19.3 ±4.0	15.7 ±3.2	13.3 ±2.8

Means±SD; slope mmHg/ml.min^{-1}; INT mmHg.

LIG given twice daily (8mg/100g IP) during hypoxic exposure reduced RV/LV + septum compared with vehicle (V) treatment (CH-LIG, n=7, 0.492±0.0565, vs CH-V, n=8, 0.654±0.113, $p < 0.005$ unpaired t-test). The slope of the P/$\dot{Q}$ line was unaltered by chronic treatment but INT was reduced both in normoxia (CH-LIG, n=6, 6.2±0.8mmHg vs CH-V, n=6, 7.8±3.8, NS) and hypoxia (CH-LIG 12.9±3.1 vs 18.3±4.5, $p < 0.05$).

460P EFFECTS OF ATRIAL NATRIURETIC PEPTIDE IN HYPOXIA ADAPTED RATS

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Atrial Natriuretic Peptide (ANP) causes a dose dependent relaxation in pulmonary arteries in vitro and in vivo. We have studied the effects of ANP on the pulmonary artery pressure (Pap) and pressure flow (P/Q) relationships both at baseline Pap and following hypoxic pulmonary vasoconstriction (HPV). Twelve male Wistar rats (SPF, Tucks) exposed to 10% O2 for 3-4 weeks in a normobaric environmental chamber (CH) were compared to 12 litter mate controls (C). Following pentobarbitone 60mg/kg ip anaesthesia the rats were intubated and their isolated lungs perfused via a Marlow roller pump with blood of normal haematocrit, temp 38°C, pH 7.35 to 7.45 at flow rates 20ml/min (baseline) (Emery et al, 1981). The lungs were ventilated by a Starling Ideal pump (tidal volume 3ml) with either air + 5% CO2 (baseline) or 2% O2 5% CO2 during HPV. ANP (30ng to 3µg bolus) had no effect on baseline Pap or P/Q in C rats but had significant effects in a dose dependent manner in CH. During HPV, ANP had a significantly greater ($p<0.001$) effect in CH rats.

Dose ANP in ng	30	100	300	1000	3000
Change baseline Pap in CH (mmHg)	0.5	0.8	2.9	4.1	4.1
% change in CH	2.6%	6.2%	9.5%	12.2%	12.6%
% abolition of HPV in CH	13.4	50.7	83.3	95.7	96.3
% abolition of HPV in C	3.9	23.5	51.0	67.8	76.5

P/Q lines were linear except at very low flow rates and were extrapolated to the P axis to give an intercept (I) which is the effective downstream pressure for flow ($Q \propto P\text{-}I$). Slope (S) is an indicator of tone. Both S and I were significantly greater in CH than C and values rose on HPV. ANP decreases S and I in both groups with a significantly greater effect in CH, 3µg ANP lowering S and I below the original air baseline. In CH rats ANP given during normoxic ventilation lowered both S and I in a dose dependent manner. ANP at pathophysiological concentrations may modulate pulmonary artery pressure and vascular tone and may influence right ventricular function during hypoxia.

Emery, C.J., Bee, D., Barer, G.R. (1981) Clin. Sci. 61 569-580.

461P THE VASODILATORY EFFECT OF REPEATED CGRP AND NITRATE ADMINISTRATION IN SMALL PIG CORONARY ARTERY RINGS

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Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide and a potent vasodilator in coronary artery preparations. In small coronary artery rings the vasodilation is endothelium independent (Beny et al, 1989; Franco-Cereceda & Rudehill, 1989), however, its exact mechanism of action is not known. Organic nitrates also exhibit endothelium independent relaxations in coronary artery preparations but repeated administration results in a rapid development of tolerance. The present study was aimed to investigate the vasodilatory profile, in terms of potency and development of tolerance, of CGRP against a range of nitrates in small pig coronary artery preparations (PCA).

PCA of diameter <1mm were mounted in 10ml organ baths containing Krebs ringer solution, maintained at 37 ± 1°C and gassed with 95% O_2 and 5% CO_2. The tissue was kept under a resting tension of 2g and changes in isometric tension were measured. PCAs were pre-constricted with a concentration of Ach (3 x 10^{-7}M)which gave a sub-maximal response. Four consecutive concentration response curves (CRC) to CGRP (10^{-10} - 10^{-8}M) were constructed using a dose cycle of 6mins. For the nitrates [sodium nitroprusside (SNP), glyceryl trinitrate (GTN) and isosorbide dinitrate (IDN)], three consecutive CRCs to each were obtained prior to and following a CRC to CGRP, the tissues being washed 3x and left for 30mins in between each CRC. All data collated from these experiments was expressed as a % reversal of tone induced by Ach (3 x 10^{-7}M) and EC_{50} values calculated. Development of tolerance was assessed by calculating the ratio of the EC_{50} of the last CRC compared to the first CRC for each compound, ie. the tolerance index (TI).

CGRP gave a concentration-dependent relaxation of the tissue which reached a maximum (119 ± 6%, mean ± sem, n = 5) at 1.28 x 10^{-8}M with an EC_{50} of 1.4 ± 0.3 x 10^{-9}M. Development of tolerance to CGRP was not seen after repeated administration (TI = 1.89 ± 1.42). The EC_{50} values for the nitrates, (SNP, 3.4 ± 0.8 x 10^{-7}M, n = 6); GTN, 1.2 ± 0.5 x 10^{-7}M, n = 6; IDN, 1.8 ± 0.9 x 10^{-7}M, n = 4), were significantly higher (P <0.001; paired-t test) than for CGRP. Unlike CGRP tolerance to the nitrates developed in all the tissues (TI : SNP, 23.3 ± 7.4; GTN, 14.7 ± 2.6; IDN, 10.6 ± 7.5). Furthermore, following development of tolerance to the nitrates, subsequent responses to CGRP were not affected. The data show CGRP to be significantly more potent in pig small coronary artery rings than the nitrates tested. In contrast to the nitrates, tolerance did not develop to CGRP suggesting that the mechanism of dilatation differs in each case.

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Franco-Cereceda,A. & Rudehill, A.(1989) ACTA. Physiol. Scand. 136, 575-580.

462P EFFECTS OF AH 21-132 ON THE MECHANICAL AND ELECTRICAL ACTIVITY OF ISOLATED PORTAL VEIN AND UTERINE SMOOTH MUSCLE

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AH 21-132 is a phosphodiesterase (PDE) inhibitor which exerts non-specific relaxant effects in airways and and gastrointestinal smooth muscle and increases the rate and force of atrial contraction (Bewley & Chapman,1988; Berry et al., 1989; Small et al.,1989a,b). The selectivity of AH 21-132 as an inhibitor of PDE isoenzyme types III and IV (Berry et al., 1989; Giembycz et al.,1989) may help to explain the greater potency of this agent in relaxing trachealis muscle as opposed to stimulating the atria or relaxing intestinal smooth muscle. We have now examined the effects of AH 21-132 on smooth muscle of the portal vein and uterus. AH 21-132 (1μM-1mM) suppressed the mechanical activity of rat portal vein in a concentration-dependent manner. Taking a 50% reduction in integrated mechanical activity as the half-maximal response, the pD2 value for AH 21-132 was 4.68 ± 0.19 (mean ± s.e.mean; n=6).Extracellular electrophysiological recording from both rat and guinea-pig portal vein showed that AH 21-132 reduced the amplitude but increased the frequency of spontaneous spike bursts. In some experiments AH 21-132 (1mM) tot ally suppressed the mechanical and electrical activity of these tissues. Uterine smooth muscle strips were obtained from 22-day pregnant rats and bathed by Krebs solution containing oxytocin (0.1 mu/ml). AH 21-132 (1μM-1mM) caused concentration-dependent suppression of integrated mechanical activity with a pD2 value of 5.06 ± 0.05. This effect was manifest principally as a reduction in the amplitude of phasic tension waves but their frequency was also reduced. Extracellular recording showed that the mechanical effects of AH 21-132 were accompanied by corresponding changes in the amplitude and frequency of electrical spike bursts. We conclude that the smooth muscle relaxant effects of AH 21-132 extend to vascular and uterine tissue. The relatively high potency of AH 21-132 in airways smooth muscle (Small et al, 1989a) compared with vascular or uterine smooth muscle may reflect differences in the distribution or functional importance of isoenzymes of PDE.

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463P THE EFFECT OF ETHANOL ON PORTAL VENOUS PRESSURE IN THE RAT

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Ethanol (E) causes hepatic cirrhosis, portal venous hypertension and oesophageal varices. "Binge drinking" in man may result in acute haemorrhage from either the gastric mucosa or varices. A sudden increase in portal venous pressure (Ppv) may be in part responsible for this and the present experiment examined the effect of E on Ppv in an isolated perfused rat liver preparation.

The livers of male Wistar rats (mean BW 211g (9sd)) were perfused in situ via the portal vein (perfusate vol 100 ml; flow rate 10 ml/min) (Burns et al, 1989). The Ppv and hepatic venous pressure were monitored continuously. After a 30 min stabilisation period, E (absolute) was infused (500 ul/h) for 30 min into a mixing reservoir (vol 70 ml) proximal to the portal vein. On another occasion, a continuous noradrenaline (NA) infusion (140 ng/min) was started 30 min after stabilisation accompanied by the E infusion 30 min later. Results are given as mean (sd).

Portal perfusate E concentration 30 min after starting the infusion was 227 (44) mg/100 ml (n=3). In the absence of E or NA, Ppv and Phv remained constant for at least 2 h. The effects of E and of NA are shown below.

Drug	n	Portal venous pressure (cm water) Pretreatment	30 min after starting NA	30 min after starting E
Ethanol	10	7.7 (0.8)	____	8.4 (1.7)*
NA + ethanol	12	7.3 (0.9)	9.4 (1.4)*	9.3 (1.6)

*$p < 0.01$ compared with pretreatment values

E, given alone, raised the Ppv by 7.7% (8.9) with a range of -3.6 to +30.0% E had no effect on Ppv when NA was coadministered. Neither E nor NA altered Phv. At a concentration likely to exist in the portal vein in man during "binge drinking" E caused the Ppv to rise and this is consistent with its vasospastic effect on epicardial coronary arteries (Rogers and Bove, 1989). The lack of effect of E on Ppv with NA coadministration may reflect an already maximal pressure effect on NA. E may have a direct and immediate effect on portal haemodynamics and in man this may increase the risk of gastrointestinal haemorrhage.

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464P α_1-ADRENOCEPTOR ANTAGONIST EFFECTS OF THE 5-HT_{1A} AGONIST 8-OH-DPAT

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The aim of this study was to determine the mechanism involved in 8-OH-DPAT-induced reduction in perfusion pressure in the rat isolated perfused mesenteric bed. These were removed from male AHA rats (175-200g) and perfused with aerated (5% CO_2 in O_2) McEwens solution (McEwen 1956; 2ml/min) at 37°C. After endothelial removal (sodium deoxycholic acid, 1mg/ml at 2ml/min for 50sec), perfusion pressure was increased to >50mmHg by continual infusion of a submaximal concentration of phenylephrine (PE, 10μM) in the perfusion fluid. Dose-response curves (DRC) were constructed by bolus injection of drugs; pEC_{50} values were calculated. Antagonists were included in the perfusion fluid 20min before, and during, the second DRC. Responses were expressed as %papaverine (200nmoles)-induced relaxation. 8-OH-DPAT (0.3-90nmol), flesinoxan (0.02-67nmol), ipsapirone (0.02-7nmol) or phentolamine (0.003-3nmol) caused transient, dose-related reductions in PE-tone (table 1; n=3-5) with similar maximum effects (~ 80% papaverine maximum). However, 8-OH-DPAT-induced relaxations were not modified by the 5-HT_{1A} antagonists BMY7378 (0.3μM, DR=2.1, n=3) or cyanopindolol (0.1μM, DR=2.1; n=3) 8-OH-DPAT caused no relaxation when perfusion pressure was raised with the thromboxane A_2-mimetic U46619. Therefore, it is possible that when tone was raised with PE, the effects of 8-OH-DPAT and these other agents were due to α_1-adrenoceptor blockade. Thus, α_1-adrenoceptor antagonist potency (pK_B) was determined in rabbit isolated aortic rings using PE as the agonist. The rank order of potency for causing relaxation in the rat mesentery was identical to that for α_1-adrenoceptor antagonist potency but different from that for 5-HT_{1A} binding affinity. In conclusion, the relaxant effects of 8-OH-DPAT in the rat PE-perfused mesentery most probably reflect α_1-adrenoceptor antagonist activity, resulting from the high local concentrations achieved with bolus dosing.

Table 1: Comparison of potency for causing relaxation of PE-induced tone in the mesentery with 5-HT_{1A} binding affinity and α_1-adrenoceptor antagonist potency.

Compound	Rat Mesentery pEC_{50} (-log[moles])	5-HT_{1A} binding pK_D*	α_1-antagonist pK_B
Phentolamine	10.32	5.52	7.54
Ipsapirone	9.62	7.73	6.30
Flesinoxan	8.98	8.32	5.79
8-OH-DPAT	8.24	8.74	5.00

* taken from Hoyer, 1988; Schoeffter & Hoyer, 1988, 1989

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465P ACTIVATION OF VENOUS α_2-ADRENOCEPTORS BY UK-14304 IN THE PRESENCE OF LOW EXTRACELLULAR Ca^{2+} IS ASSOCIATED WITH AN INITIAL TRANSIENT CONTRACTION

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Activation of alpha$_2$-adrenoceptors by noradrenaline (NA) and UK-14304 in human erythroleukaemia cells (HEL) and by NA in the rabbit isolated ear vein is linked to the release of cellular-bound Ca^{2+} ions (Michel *et al.*, 1989; Daly *et al.*, 1990). In view of the many differences between phenethylamine and imidazoline alpha-adrenoceptor agonists (McGrath *et al.*, 1989), we have examined the role of cellular-bound Ca^{2+} in alpha$_2$-adrenoceptor-mediated contraction of vascular smooth muscle elicited by UK-14304.

Ring segments of rabbit isolated ear vein, plantaris vein and saphenous veins were prepared for isometric tension recording as previously described (Daly *et al.*, 1990). Responses to 3μM NA or 3μM UK-14304 were elicited in Krebs-Henseliet (K-H) solution containing either 2.5mM (normal) or 0.1μM (low Ca^{2+}) 'free' Ca^{2+} ions, and those to 3μM UK-14304 in low Ca^{2+} K-H solution were repeated in either the absence or presence of 0.1μM prazosin or 1μM rauwolscine.

In normal K-H solution responses to UK-14304 in each vessel, like those to NA, were sustained. After a 10min exposure to low Ca^{2+} solution, 3μM UK-14304 produced an initial transient contraction (ITC); an increase in tension within 30sec followed by relaxation towards baseline. The ITC to 3μM UK-14304 in the ear vein was similar to that produced by 3μM NA (30±3.4% of the NA response in normal K-H solution, n=5). In the plantaris vein and saphenous vein, however, the ITC to UK-14034 were significantly less than those produced by 3μM NA. 1μM rauwolscine significantly reduced the ITCs to 3μM UK-14304 (> 80%) in all three preparations. 0.1μM prazosin failed to reduce the ITC to 3μM UK-14304 in the ear and plantaris veins by more than that observed in control preparations (< 25%), but abolished the ITC to 3μM UK-14304 in the saphenous vein.

Low Ca^{2+} K-H solution abolishes responses to KCl and prevents the Ca^{2+} entry necessary to support contraction (Daly *et al.*, 1989). Thus, alpha$_2$-adrenoceptors in the ear and plantaris veins, like those on HEL cells (Michel *et al.*, 1989), do not appear to discriminate between imidazoline and phenethylamine agonists in relation to the release of cellular-bound Ca^{2+} ions.

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466P INHIBITION OF NORADRENALINE-INDUCED HYDROLYSIS OF PHOSPHATIDYLINOSITOL BY cAMP AND cGMP IN RAT ISOLATED AORTA.

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In vascular smooth muscle, noradrenaline (NA)-induced contractions, mediated via alpha$_1$-adrenoceptors, are dependent on hydrolysis of phosphatidylinositol (PI). Such contractions can be inhibited by sodium nitroprusside (SNP), which activates guanylate cyclase and increases synthesis of cyclic guanosine monophosphate (cGMP). How increased cGMP synthesis inhibits contraction is unknown but it is likely that vasorelaxants which increase cGMP synthesis will inhibit PI hydrolysis. This study examined this possibility by investigating the effects of SNP, and of the permeant cyclic nucleotide, 8-Br-cGMP. In addition, the possibility that cyclic adenosine monophosphate (cAMP) might also inhibit PI hydrolysis was investigated using the adenylate cyclase activator, forskolin (Lincoln & Fisher-Simpson, 1983) and 8-Br-cAMP.

The effects of NA (6.2 μM) (alone and in combination with SNP (μM), 8-Br-cGMP (0.3 mM), forskolin (10 μM) or 8-Br-cAMP (0.3 mM)) on PI hydrolysis were investigated in rat aortic rings (2-3 mm), pre-labelled with myo-^{3}H-inositol (incubation: 37°C, 8 μCi/ml, 2 hr). Aortic rings were incubated with drug(s) (37°C, 1 hr) in Krebs buffer containing lithium chloride (10 mM). The reaction was stopped by adding a mixture of chloroform and methanol (1:2 v/v). Aortic rings were then homogenised using a Potter-Elvehjem homogeniser and the inositol phosphates were extracted and separated on Dowex resin using a gradient of ammonium formate in formic acid (100 mM). Radioactivity was measured by liquid scintillation counter.

NA increased the accumulation of ^{3}H-inositol phosphates in aortic rings (from 72.9 ± 1.5 (n=11) to 637.5 ± 27.9 (n=11), dpm mg^{-1}, mean ± s.e. mean $P < 0.001$). This response to NA was inhibited by SNP from 637.5 ± 27.9 (n=11) to 351.5 ± 17.1 (n=17), $P < 0.001$) or 8-Br-cGMP (from 637.5 ± 27.9 (n=11) to 357.9 ± 12.6 (n=16), $P < 0.001$) and also by forskolin from 637.5 ± 27.9 (n=11) to 334.5 ± 23.9 (n=12), $P < 0.001$) or 8-Br-cAMP (from 638.0 ± 13.4 (n=9) to 338.5 ± 35.8 (n=9), $P < 0.001$). These results indicate that in rat aorta, the NA-induced PI response can be inhibited by SNP, which increases cGMP synthesis and by forskolin, which increases cAMP synthesis. The similar inhibitory effects of the permeant cyclic nucleotide derivatives, 8-Br-cAMP, suggest that drugs which increase the synthesis of cGMP or cAMP in vascular smooth muscle, may owe their vasorelaxant activity, at least in part, to the inhibitory effect of these nucleotides on PI hydrolysis.

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467P 5-HT-INDUCED DILATATION IN THE RAT TAIL VASCULATURE

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5-hydroxytryptamine (5HT) produces constriction of the rat caudal tail artery (Van Neuten et al. 1981), but there is little information on whether 5HT influences other components of the tail vasculature, or indeed if more than one type of 5HT receptor is present. The effect of 5HT has therefore been studied in vivo in the perfused rat tail circulation, isolated from the systemic supply, but with the nerve supply intact.

Experiments were carried out on 35 anaesthetised, heparinised, male Wistar rats (400-500gr, 120mg Kg^{-1} thiopentone i.p. plus 20mg hr^{-1} i.v.). The tail circulation was isolated by ligatures placed round the lateral and ventral arteries and veins at the level of the fifth caudal vertebra and the mid-ventral artery within the abdomen. The caudal artery was cannulated, all veins incised distal to the sutures and the tail vasculature perfused with Krebs/Henseleit solution (1.1ml min^{-1}, pH 7.35, 37°C) to which Ficoll 70 (20gr l^{-1}) had been added (Key et al. 1983). Drugs were added to the perfusate as 0.1ml bolus injections. Systemic BP, heart and respiratory rates remained unaltered. 5HT was studied in: 1. Normothermic animals (body temp. 37-37.5°C). 2. Normothermic animals initially subjected to periods of high perfusion rates (>4ml min^{-1}), coupled with 0.1-0.2ml air injections to induce loss of vascular endothelial lining (Ralevic et al. 1989). 3. Dead animals in which the injection of microspheres (15-20μm diam.) indicated extensive opening of arterio-venous anastomoses (AVA's).

In normothermic animals 5HT (3.4pmol-3.4nmol) produced dose-related dilatations (10-40mm Hg), superimposed on which, at doses above 68.5pmol, were constrictor responses (10-150mm Hg). These effects were duplicated by (±)α-methyl-5-hydroxytryptamine (α-Me5HT) at similar dose levels (5pmol-5nmol). 1(m-Chlorophenyl)-piperazine (mCPP, 16.5pmol-3.7nmol) produced only dilatations, as did 5-carboxamidotryptamine (5CT, 27.4pmol-13.7nmol) but at doses 8-10 times higher than those of 5HT and mCPP. Dilatation induced by 5HT, α-Me5HT, mCPP, 5CT as well as ACh (1nmol-11nmol) was abolished in type 2 preparations even though the dilatatory effect of isoprenaline remained unchanged. In type 3 preparations the dilatatory effects of all drugs were abolished but constrictor responses to 5HT and α-Me5HT were enhanced (40-60%) and appeared with doses as low as 17pmol. 5CT now produced small constrictions (5-25mmHg) but only with high doses (>1.4nmol).

In the rat tail vasculature 5HT produces both constriction and dilatation. The dilatation could be related to an endothelial receptor, which on the basis of agonist responses, would appear to be similar to the $5HT_{1C}$ subtype. 5CT did not appear to be an effective constrictor of AVA's within the tail vasculature.

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468P RECEPTOR-MEDIATED ADENYLATE CYCLASE INHIBITION AND CELLULAR RESPONSES

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The relationship between increases in intracellular cyclic AMP (cAMP) concentrations and functional responses, such as stimulation of adipocyte lipolysis or relaxation of vascular smooth muscle, has been studied extensively. In general, such studies have concluded that the concentration-effect curves for elevation of tissue cAMP concentrations lie to the right of those for the corresponding functional end-response (e.g. Sumner et al, 1989). In this study, we have explored the relationship between decreases in cAMP concentrations and two functional end-responses, namely adenosine A_1 receptor-induced inhibition of lipolysis and $5\text{-}HT_1$-like receptor-induced contraction of vascular smooth muscle.

Human adipocytes were isolated by collagenase digestion of abdominal fat, removed during routine surgery, essentially as described by Stratton et al (1985). The adipocytes were preincubated (37°C, 15-30 min) with varying concentrations of 5'-N-ethylcarboxamidoadenosine (NECA) before being exposed to 10^{-5}M or $3x10^{-8}$M (-) isoprenaline (37°C; 2 min or 2 hours respectively) to stimulate cAMP formation or lipolysis. Rings (2-3 mm) of dog isolated saphenous vein were coincubated (2 min, 37°C) with 5μM prostaglandin E_2 (PGE_2) and a range of concentrations of the $5\text{-}HT_1$-like receptor agonist, sumatriptan (GR43175, Humphrey et al, 1988) and used for the measurement of tissue cAMP (Sumner et al, 1989).

NECA was found to be equipotent in its ability to inhibit both isoprenaline-induced lipolysis and cAMP accumulation, the geometric mean EC_{50} values (with 95% CLs) being 14(3-65)nM and 20(11-34)nM respectively (n=5). We have recently shown that sumatriptan inhibits PGE_2-stimulated cAMP accumulation in the dog isolated saphenous vein with an EC_{50} of 250nM (Sumner & Humphrey, 1990). Sumatriptan has previously been reported to contract this tissue with a comparable EC_{50} of 302nM (Humphrey et al, 1988).

These results show that concentration-effect curves for responses evoked by two agonists acting at different receptors which are negatively coupled to adenylate cyclase, overlay the concentration-effect curves for the inhibition of cAMP formation in the same tissues. This contrasts markedly with observations in systems in which responses result from activation of adenylate cyclase.

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469P MODULATION OF Na^+/K^+-ATPase IN CULTURED BOVINE AORTIC ENDOTHELIAL CELLS BY GLUCOSE AND GALACTOSE

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Peripheral vascular insufficiency is a serious complication of diabetes mellitus. The present study represents the beginning of an exploration of the role of disordered endothelial cell function in the development of this phenomenon. Analogous work on diabetes-induced nerve dysfunction indicates that glucose induces aberrant modulation of Na^+/K^+-ATPase activity. Furthermore, this phenomenon may involve exaggerated flux through the polyol pathway via the enzyme aldose reductase (Greene *et al.*, 1988). Accordingly, we have studied the effects of the polyol pathway substrates, glucose and galactose, on Na^+/K^+-ATPase in cultures of bovine aortic endothelial cells. Cells were released from fresh aortae by luminal scraping and digestion in 0.02% collagenase. Cells were cultured on gelatine-coated dishes in Eagles MEM containing 10% fetal calf serum. When confluent, the cells were passaged with 0.05% trypsin/0.01% EDTA, replated onto 30mm dishes and left for 24h. Cells were then transferred to normal medium (containing 5mM glucose) or medium supplemented with 10 mM galactose or with 20 mM glucose. These conditions were maintained, with changes of medium every 48h, for 7 days. Uptake of [^{86}Rb] was then measured in the absence and presence of ouabain (0.2 mM) and Na^+/K^+-ATPase activity was calculated by subtraction. Incubation with 10 mM galactose was associated with a reduction of Na^+/K^+-ATPase activity of 40% with respect to control cells ($P < 0.01$). On confluent monolayers elevated glucose was also associated with a reduction in Na^+/K^+-ATPase activity - by 40% relative to controls ($P < 0.01$). These findings indicate a connection between polyol pathway flux and Na^+/K^+-ATPase in cultured endothelial cells, but that the effect may be masked during high glucose utilisation in the growth phase.

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470P THE EFFECTS OF A LOW EXTRACELLULAR CONCENTRATION OF POTASSIUM FOR 72 h ON Na/K PUMP NUMBERS AND ACTIVITY IN HUMAN LYMPHOBLASTS AND THEIR REVERSAL WITHIN 24 h

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We have previously reported the effect of reducing the extracellular potassium concentration (low K) on [^{3}H]-ouabain binding and ^{86}rubidium (Rb) influx in human lymphoblasts (Kennedy et al., 1987). We now report the effects of returning lymphoblasts from a low K medium to a normal K medium. Lymphoblasts were grown in RPMI 1640 and 10% dialysed fetal calf serum with 10^5 iu/l penicillin and 100 mg/l streptomycin at 37°C and 100% humidity in air/CO_2 (95%/5%). Two sets of lymphoblasts were grown in either 5 mM or 0.5 mM K for 72 h. One set of cells was then washed and resuspended in 5 mM K for a further 24 h. Specific [^{3}H]-ouabain binding and ^{86}Rb influx were measured at 72 h and at 96 h as previously described (Oh et al., 1987). Binding was measured at seven concentrations of [^{3}H]-ouabain from 5 to 40 mM; K_D and B_{max} were calculated from Scatchard plots. Non-specific binding, estimated with 10^{-4} M non-radioactive ouabain, was less than 1% of total. ^{86}Rb influx was measured at six concentrations of RbCl from 0.6 to 6 mM, supplemented with 1 µM ^{86}Rb; K_m and V_{max} were determined assuming two external binding sites for Rb (Garay and Garrahan, 1973).

The results are shown in Table 1. Low K (0.5 mM) caused significant increases in the B_{max} of [^{3}H]-ouabain binding (and therefore, by inference, in the number of Na/K pumps in the membrane), the K_D of [^{3}H]-ouabain binding, and the V_{max} and K_m of Rb influx. The turnover rate of ions per site did not change. The increases in the K_D of [^{3}H]-ouabain binding and the K_m and V_{max} of Rb influx were completely reversed within 24 h. There was partial reversal of the B_{max} of [^{3}H]-ouabain binding by 24 h ($P=0.06$).

Table 1. Recovery by lymphoblasts from the effects of low K. [The results are given as mean (sd); *P<0.05 or lower vs control 72 h; + P=0.06 vs control recovered.]

	K_m (mmol/l)	K_D (nmol/l)	V_{max} (fmol/cell/min)	B_{max} (fmol/10^6 cells)	Sites/cell	Turnover (ions/site/min)
Control 72 h	0.62 (0.20)	20.1 (3.5)	3.96 (1.67)	1769 (494)	1.06×10^6	2380
Low K 72 h	0.81 (0.25)*	23.5 (4.2)*	5.98 (1.94)*	2563 (701)*	$1.54 \times 10^{6*}$	2338
Control recovered	0.69 (0.24)	19.8 (3.7)	4.66 (1.78)	1700 (340)	1.02×10^6	2750
Low K recovered	0.71 (0.23)	20.7 (3.4)	4.44 (1.53)	1970 (426)+	1.16×10^6	2310

These results confirm our earlier findings on the effects of low K for 72 h on Na/K pump numbers and activity in lymphoblasts. However, the reversibility of most of these effects was faster than their onset, since the effect of low K on [^{3}H]-ouabain binding does not occur within 24 h (Kennedy et al., 1987). Nevertheless, the reversal of the increase in Na/K pump numbers was not complete at 24 h, and this suggests that some of the pumps which are newly formed in response to low K may not be active in transporting Na and K. This interpretation is also consistent with the observation that low K for 72 h increases the K_D of [^{3}H]-ouabain binding and the K_m of rubidium influx.

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471P CALCIUM-DEPENDENT RELEASE OF EICOSANOIDS IN HUMAN CULTURED BRONCHIAL EPITHELIAL CELLS

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Epithelial cells are implicated in the pathogenesis of airways inflammation, either by releasing inflammatory agents themselves or being the target of leucocyte-induced injury. Although the pathways of arachidonic acid metabolism have been investigated in human tracheal epithelium (Holtzman et al., 1988), little is known about the capacity of human bronchial epithelial cells to release pro-inflammatory lipid mediators. We have investigated this question using human bronchial epithelial cells grown in culture.

Ciliated and differentiated non-ciliated human bronchial epithelial cells were grown in explant culture and characterized by positive staining with the monoclonal antibodies CAM 5.2 (cytokeratin) and PR1A3 (cilia). Initially, the routes of arachidonic acid metabolism were established by radiolabelling with tritiated arachidonic acid. In the subsequent studies reported here quantitative analyses were performed by RIA. Calcium ionophore A23187 (0.5-20 μM) induced a concentration-dependent release of prostaglandin (PG) E_2, with maximum release occuring 20 min after challenge. To avoid possible cytolytic effects of the ionophore, challenges were routinely performed with 2.5 μM A23187 for 20 min. The profile of eicosanoid release is shown below.

	Immunoreactive Eicosanoid Release (pg μg^{-1} protein)			
	PGE_2	LTB_4	6-keto-$PGF_{1\alpha}$	15-HETE
Control	2.21 ± 0.75	6.82 ± 3.25	2.40 ± 0.91	40.78 ± 17.93
A23187	7.41 ± 2.23*	4.61 ± 0.84	2.26 ± 1.19	39.57 ± 16.08

*$P<0.05$ with respect to control. Mean ± s.e. mean from 4-9 experiments.

PGE_2 was the major cyclooxygenase product released in response to A23187 challenge. The large amount of 15-HETE release which occurred spontaneously was not further increased by the ionophore. The presence of this hydroxy acid has previously been reported in tracheal cells challenged with arachidonic acid (Holtzman et al., 1988).

In 4 experiments PGE_2 at up to 1μM was without significant effect on the permeability of bovine bronchial epithelium to ^{125}I-labelled albumin (control permeability 1.92 ± 0.22 fmol $cm^{-2}min^{-1}$ compared to 1.90 ± 0.16 fmol $cm^{-2}min^{-1}$ at 1μM). The function of PGE_2 derived from epithelial cells may thus be to act as an immunomodulator rather than acting as an agent affecting the structural integrity of the epithelium.

Holtzman, M.J., Hansbrough, J.R., Rosen, G.D., Turk, J. (1988) Biochim. Biophys. Acta **963**, 401-413.

472P DOES THE GUINEA-PIG TRACHEA CONTINUOUSLY RELEASE A SMOOTH MUSCLE RELAXING FACTOR?

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The search for a novel relaxing factor from airway epithelium (EpDRF) has focussed on observing its effects after stimulation by various receptor ligands. Using a modification of the co-axial bioassay developed by Guc et al (1988), we have investigated the apparently continuous release of a relaxant activity from guinea pig trachea.

Anococcygeus muscles (RA) from male rats (250-400g) were suspended under 1g tension in Krebs-Henseleit solution aerated with 95%O_2/5%CO_2 (KH) and contracted with 10μM phenylephrine (PE). Changes in muscle length were measured using auxotonic levers. Tracheae (GPT) from male guinea pigs (500-750g) were slit along the trachealis muscle and immobilised inside a piece of silicone tubing (length 30mm, i.d. 4.5mm). This was positioned so that it could be lowered over the RA without touching it. The epithelium was removed from selected GPTs. In some experiments, the partial pressure of oxygen (pO_2) of the KH inside and outside the GPT was measured using a Neocath O_2 electrode (Biomedical Sensors).

When lowered over the RA, the GPT caused reversible relaxation of the muscle. The degree of relaxation was proportional to the length of GPT; no relaxation was seen if the GPT was first killed by boiling. The response was unaffected by indomethacin (2.8μM), superoxide dismutase (100iu/ml) or L-NG monomethyl-arginine (300μM); neither could it be mimicked by glyceryl trinitrate (4.4μM), bradykinin (10μM) or sodium nitroprusside (100nM). No further relaxation was seen when acetylcholine (1μM-1mM) was added to the preparation. The effect of epithelial removal was variable; the relaxation was attenuated or abolished in 5 out of 11 experiments. We have shown that the reduced pO_2 inside the GPT is at least partly responsible for the relaxant activity seen in co-axial bioassays (Gunn & Piper, 1990). The pO_2 inside a whole GPT tied into a silicone tube was 302 ± 18mmHg (512 ± 31mmHg outside). This length of GPT caused the RA to relax to 38 ± 5% of the initial response to PE, which is a significantly greater response than occurred when the pO_2 of the KH was reduced to a similar value (86 ± 2%) ($p<0.05$).

It seems improbable that the GPT-induced relaxation of RA is entirely due to reduced O_2 availability. However, the activity observed is neither a cyclooxygenase product nor is it related to EDRF or even to the postulated EpDRF.

We thank the MRC for financial support.

Guc, M.O., Ilhan, M. & Kayaalp, S.O. (1988), Eur. J. Pharmacol. 48.405-409
Gunn,L. & Piper,P.J. (1990) This meeting.

473P INHIBITION OF NEURALLY MEDIATED CONTRACTION OF GUINEA-PIG AIRWAY SMOOTH MUSCLE BY FRUSEMIDE AND BUMETANIDE *IN VITRO*

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Frusemide, a loop diuretic, has recently been shown to inhibit the responses to a wide variety of bronchoconstrictor stimuli such as allergen, exercise, sodium metabisulphite and adenosine in asthmatic subjects (Bianco *et al*, 1989; Nichol *et al*, 1990), but is not effective against methacholine- or histamine-induced bronchoconstriction. We have investigated the effect of frusemide on neurally-mediated airway smooth muscle contraction, and compared its effects to those of bumetanide, another clinically more potent loop diuretic, which also inhibits an electroneutral $Na^+/K^+/2Cl^-$ co-transporter protein in the kidney tubule.

Guinea-pig tracheal and bronchial strips were prepared and suspended between platinum plate electrodes in organ baths containing Krebs-Henseleit solution bubbled with 95% O_2, 5% CO_2. Tissues were equilibrated under an initial basal tension of 1.5 g in the presence of indomethacin (10 μM) and propranolol (1 μM). Bronchial strips were also incubated with atropine (1 μM). Contractile responses were measured with Grass force-displacement transducers. Biphasic square wave pulses were delivered to the electrodes (20V: 0.5ms pulse duration: 0.5-50 Hz for 20 s). For each tissue a baseline response to electrical field stimulation (EFS) was performed, followed by a repeat response after incubation with either frusemide or bumetanide.

Frusemide (1 and 0.1 mM) caused significant inhibition of the response to EFS at all frequences, the maximal effect being at 4 Hz (42 ± 5% and 41 ± 6% respectively, $P < 0.01$). Bumetanide (1 - 100 μM) was more potent with significant effects seen at lower stimulation frequences. At 4 Hz, 100 μM bumetanide produced the maximal observed inhibition of the stimulation-induced contraction, (57 ± 4%, $P < 0.01$), and with only 26 ± 4% inhibition at 50Hz. In the hilar and main bronchi, frusemide and bumetanide caused a concentration-dependent inhibition of non-adrenergic, non-cholinergic mediated contraction induced by EFS at 8 Hz. Bumetanide was more potent than frusemide giving 66 ± 5% and 36 ± 6% inhibition at 10 μM respectively. Neither diuretic had a significant effect on acetylcholine- or substance P-induced contraction of tracheal and bronchial strips. Epithelial removal by gentle luminal rubbing (confirmed histologically) did not significantly alter the inhibitory effect of frusemide in the trachea.

The results suggest that frusemide and bumetanide inhibit neurally induce contraction if guinea-pig airway smooth muscle *in vitro* by inhibiting the release of neurotransmitters from cholinergic and non-cholinergic sensory motor nerves. The potencies of frusemide and bumetanide correlate well with their known loop diuretic properties and their inhibitory effect on the $Na^+/K^+/2Cl^-$ co-transporter protein.

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Nichol, G., Alton, E.W.F.W., Nix, A., Geddes, D.M., Chung, .F. & Barnes, P.J. (1990). Am.Rev.Resp.Dis., *in press*

474P PREFERENTIAL PRE-JUNCTIONAL SITE OF INHIBITION OF NON-CHOLINERGIC BRONCHOSPASM BY POTASSIUM CHANNEL OPENERS (KCOS)

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In guinea-pig tracheal preparations in vitro cromakalim was more effective in reducing contractions induced by cholinergic nerve stimulation than those induced by exogenously added transmitter, suggesting a pre-junctional action preventing transmitter release (Hall & MacLagan, 1988; McCaig & De Jonckheere, 1989). In addition to the cholinergic pathway there is a non-cholinergic excitatory innervation to the airways stimulation of which results in bronchospasm. It has been suggested that Substance P (SP) is the transmitter involved (Barnes, 1986). It has been shown (Ichinose & Barnes, 1990) that cromakalim more potently inhibits responses to non-cholinergic nerve stimulation than responses to exogenous SP. Here we have compared the KCOs cromakalim, RP49356 and its (-)enantiomer RP52891 against non-cholinergic nerve stimulation (NS) and exogenously applied SP.

Guinea pigs were anaesthetized with pentobarbitone sodium (60 mg/kg, i.p.) and artificially ventilated. All experiments were performed following administration of atropine (1mg/kg i.v.) to remove the influence of the cholinergic system. The vagus was stimulated (7V, 10Hz, 5ms) for 30 s to produce a 60 - 70% maximum bronchospasm, assessed as the increase in pulmonary inflation pressure. SP (i.v. bolus) was titrated to produce responses of the same magnitude to NS to allow direct comparison of the KCOs against each parameter. KCOs were administered as nebulized aerosol solutions.

Table 1 Effect of KCOs on bronchoconstrictor responses to NS and SP in the anaesthetized guinea pig

Compound	Dose (μg/ml/1 min)	n	% inhibition of bronchospasm NS	SP
cromakalim	500	6	65 ± 3	20 ± 9
RP49356	1000	4-6	64 ± 8	31 ± 11
RP52891	500	6	58 ± 5	23 ± 9

These results confirm and extend those of Ichinose & Barnes (1990) demonstrating that in vivo the KCOs are more potent inhibitors of non-cholinergic nerve stimulation than of exogenously added transmitter. This suggests that the KCOs are preferentially acting pre-junctionally to prevent transmitter release.

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Hall, A.H. & MacLagan, J. (1989) Br. J. Pharmac. 95, 792P.
Ichinose, M. & Barnes, P.J. (1990) J. Pharmac. Exp. Ther. (in press).
McCaig, D.J. & De Jonckheere, B. (1989) Br. J. Pharmac. 96, 252P.

475P EFFECTS OF AH 21-132, THEOPHYLLINE, FORSKOLIN AND SODIUM NITRITE ON THE CYCLIC NUCLEOTIDE CONTENT OF GUINEA-PIG ISOLATED TRACHEALIS

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Strips of guinea-pig trachea were incubated for 15 min in the absence or presence of various drugs. Frozen tissues were pulverised prior to the extraction and radioimmunoassay of cyclic nucleotides. Tissue protein was assayed by the Bradford (1976) method. The results of these experiments are presented in table 1.

Table 1

Treatment	cAMP	cGMP	Treatment	cAMP	cGMP
Control	11.4±1.0 (23)	0.88±0.07 (18)	Forskolin		
AH 21-132			160nM	7.2±0.5 (5)	---
1μM	11.6±1.2 (14)	1.23±0.15 (9)	344nM	28.6±3.1 (5)*	0.90±0.13 (5)
10μM	14.2±1.5 (13)	1.24±0.17 (8)	742nM	67.9±8.7 (8)*	1.21±0.21 (10)
30μM	24.4±3.3 (4)*	1.74±0.23 (3)	1600nM	313.3±86.2 (5)*	1.20±0.19 (4)
100μM	27.9±3.4 (12)*	2.84±0.43 (8)*	Sodium nitrite		
1mM	41.2±4.6 (8)*	3.07±0.24 (10)*	2.5mM	9.3±1.1 (9)	0.78±0.11 (4)
Theophylline			25mM	14.4±1.0 (15)*	2.16±0.23 (15)*
0.1mM	12.5±1.4 (5)	---	AH 21-132 1μM		
1mM	14.8±1.9 (9)	1.11±0.08 (4)	+	75.3±6.3 (4)*	1.24±0.40 (4)
10mM	50.8±4.9 (8)*	1.51±0.18 (9)*	Forskolin 160nM		

Cyclic nucleotide content in pmol/mg protein (mean ± s.e. mean). Figures in parentheses indicate number of experiments. *indicates a significant increase compared with controls.

The greater potency of AH 21-132 in increasing cAMP content as opposed to cGMP content is consistent with its proposed (Berry et al., 1989) selective inhibition of PDE isoenzyme types III and IV. The ability of a combination of individually-subthreshold concentrations of AH 21-132 (1μM) and forskolin (160nM) to increase tissue cAMP content suggests that the influence of AH 21-132 in preventing cAMP breakdown is best observed as changes in the tissue content of cAMP when cAMP production is stimulated by the activation of adenylate cyclase.

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cardiovascular responses to the K+ channel activator lemakalim (BRL38227)

476P TEMPORAL DIFFERENCES IN THE EFFECT OF GLIBENCLAMIDE ON PULMONARY AND CARDIOVASCULAR RESPONSES TO THE K+ CHANNEL ACTIVATOR LEMAKALIM (BRL38227)

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Glibenclamide (Glib), a blocker of ATP-sensitive potassium channels (K_{ATP}) has been shown to antagonise the vasorelaxant effects of lemakalim (BRL 38227) in vitro (Wilson et al, 1989), and to reduce the inhibitory effect of lemakalim on bronchoconstriction induced by vagal stimulation in vivo (Ichinose and Barnes, 1989). In this study we have investigated the effects of Glib on the vasorelaxant and anti-bronchoconstrictor effects of lemakalim in anaesthetised guinea-pigs.

Airway resistance (R_{AW}), dynamic lung compliance (C_{DYN}) and systemic blood pressure (BP) were measured as described previously (Bowring et al, 1989). Bronchoconstriction was induced by histmaine (i.v.) and responses were elicited every 10min. Lemakalim (50μg kg^{-1}i.v.) was given 2min prior to histamine challenge, and changes in BP were measured immediately prior to challenge. Glib (20mg kg^{-1}i.v.) was given 15 or 30min prior to lemakalim.

Lemakalim did not alter baseline levels of R_{AW} or C_{DYN} (n = 6); but reduced BP by 38±2.5% ($P<0.001$). This dose reduced the subsequent histamine-induced changes in R_{AW} and C_{DYN} by 45.0 ±10.7% ($P<0.01$), and 47.9+-6.75%($P<0.01$) respectively. Glib had no effect on airways function, but BP was was increased by 37.5+-2.5%($P<0.001$) 5min after dosing. Given 15min prior to lemakalim (n = 5), Glib reduced the effects of lemakalim to 1.32±5% on R_{AW}, and 0.4±5.5% on C_{DYN}. However, Glib given 30min prior to lemakalim (n = 5), did not alter the effects of lemakalim on histamine-induced bronchoconstriction. In contrast, Glib reduced the hypotensive effects of lemakalim to 22.8±3.7%($P<0.01$) at 15min post dose, and to 18.4±3.5% ($P<0.01$) 30min post dose.

These results may be explained by differences in the pharmacokinetics of Glib in the lung and blood vessels. Alternatively, there may be two populations of K_{ATP}, witn differing sensitivities to Glib in the lung and cardiovascular system. However, there was no difference in the responses to lemakalim and Glib of the K_{ATP} of the conducting airways (R_{AW}), and those of the smaller airways and lung parenchyma (C_{DYN}), suggesting that the population of K_{ATP} in the lung is homogeneous.

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Ichinose, M. and Barnes, P.J. (1990) J. Pharm. Exp. Therap. (in press).
Wilson, C., Hamilton, T.C., and Cawthorne, M.A. (1989) Br. J. Pharmacol., 98, 723P.

477P *IN VITRO* TECHNIQUES FOR THE STUDY OF TRANSEPITHELIAL PROTEIN FLUX IN THE AIRWAYS AND ITS MODULATION BY INFLAMMATORY CELLS AND MEDIATORS.

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In diseases such as bronchial asthma there is extensive disruption of the airway mucosa and evidence of the abnormal presence of plasma proteins in airway surface lining fluid and mucus plugs. The mechanism of these changes is not established, although an extensive repertoire of chemical mediators has been implicated as effectors. In order to investigate possible mechanisms of epithelial injury we have been developing *in vitro* techniques to study the transepithelial flux of proteins.

Bovine lungs were obtained freshly from a local abattoir and the bronchial and tracheal mucosae removed by dissection to yield sheets of tissue consisting of the airways epithelium and basement membrane. By micrometry tracheal and bronchial tissue thicknesses were 0.173 ± 0.003 mm (n=42 animals) and 0.083 ± 0.001 mm (n=70 animals) respectively. These were mounted between the two halves of specially-constructed heated (37°C) chambers which were filled with HEPES-buffered Eagle's minimum essential medium. The net unidirectional (basolateral-apical) flux of protein was determined using ^{125}I albumin (BSA) over a 180 min period. In some experiments, ^{3}H mannitol was used as an additional marker. Tracers were added to the basolateral side of the tissue and samples taken at timed intervals from the apical side of the epithelium. Radioactivity determination was performed in a gamma counter or by liquid scintillation counting employing a spectrum unfolding technique for the simultaneous quantification of ^{3}H and ^{125}I.

In the tracheal mucosa the net basolateral-apical fluxes of BSA and mannitol were 1.2 ± 0.1 and 9.3 ± 0.6 fmol $cm^{-2}min^{-1}$ respectively (n=31) when the concentration of albumin on the basolateral side was 0.4μg ml^{-1} (1μCi ml^{-1}). For individual preparations, this difference in rates between the two markers reflects the approximate 10-fold difference in their molecular radii (3.8 versus 0.4 nm). The net flux of BSA was linearly (r=0.70, n=30, P<0.001) related to the starting concentration over the range 0.25-4.0 mg ml^{-1}. With the bronchial mucosa the net basolateral-apical flux of BSA was 2.4 ± 0.2 fmol $cm^{-2}min^{-1}$ (n=22) when a starting concentration of 0.4μg ml^{-1} was used. Compared with the trachea, the higher value probably reflects the differences in tissue thickness between the two sites. Addition of leukotrienes (LT) B_4, C_4, D_4 or E_4 at a concentration of 1 x 10^{-7}M to the basolateral side of the tissue failed to produce a significant change in BSA flux in either tracheal or bronchial mucosa. In the trachea, control flux was 1.5 ± 0.2 fmol $cm^{-2}min^{-1}$ compared with values of 2.2 ± 0.5, 1.6 ± 0.2, 1.7 ± 0.2 and 1.8 ± 0.3 in the case of each of the LTs (n=4). In contrast, addition of guinea pig eosinophils to the basolateral side of the tissue, followed by their activation by either A23187 or an IgG-dependent mechanism, produced significant changes in bronchial, but not tracheal, permeability to BSA (Herbert *et al.*, This Meeting).

Herbert, C.A., Edwards, D., Boot, J.R. & Robinson, C. (1990). This meeting.

478P ELECTRICALLY EVOKED SHORT CIRCUIT CURRENT RESPONSES IN HUMAN ISOLATED COLONIC MUCOSA ARE REDUCED BY LOPERAMIDE BUT NOT BY MORPHINE

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In vivo investigations in man ascribe the anti-diarrhoeal actions of codeine and morphine to an enhancement of non-propulsive activity (Schiller et al, 1982; Kaufman et at, 1988). For the opiate analogue loperamide both changes in motor function (Schiller et al, 1984) and rate of absorption (Hughes et al, 1983; Kachel et at, 1983) have been proposed. Data from numerous animal studies show net absorption of water and electrolytes to be an important component of opioid anti-diarrhoeal action (for review see Kromer, 1988). The purpose of the present investigation was to determine whether morphine and loperamide could directly affect electrogenic ion transport mechanisms in human sigmoid colonic mucosa. Sheets of mucosa plus submucosa were set up in Ussing chambers. With this method any changes in mucosal transport will be independent of changes in motility and/or blood flow. Values of short circuit current (s.c.c.) quoted are for an exposed membrane area of 0.64cm^2.

After 60min, basal s.c.c. was 84 ± 10µA (n=35). Electrical field stimulation (EFS, 10H_3 1ms for 15s) of mucosal preparations gave voltage dependent increases in s.c.c. ranging from 12 ± 4 µA at 5v (1.8mA, n=10) to 36 ± 5 µA at 40v (30mA, n=24). TTX (3.1µm) abolished responses to EFS and reduced basal s.c.c. levels by 27 ± 8% (P<0.05, n=8). Morphine (26.4µm) had no effect on responses to EFS (10 or 40v, P>0.05, n=6) or on basal s.c.c. (P>0.05, n=9). Loperamide (19.5µm) reduced responses to EFS (10 or 40v) by 77 ± 7% (P<0.05, n=5) . The effects of loperamide were not abolished by naloxone (2.8µm, n=2). That basal s.c.c. could be lowered in these preparations was demonstrated by the actions of noradrenaline (3.0µm, n=3) which reduced basal s.c.c. from 72 ± 4µA to 54 ± 7µA.

In conclusion basal s.c.c. or electrically induced changes in s.c.c. were unaffected by morphine. Loperamide antagonised electrically induced changes in s.c.c. but probably through non-opioid mechanisms. Such non-opioid effects of lopeamide have also been observed using rat colonic mucosa. (Diener et al, 1988).

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479P THE EFFECTS OF IDAZOXAN ON FLUID TRANSPORT AND TRANSIT IN RAT COLON *IN VIVO*

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The effects of idazoxan (RX781094), a predominantly α_2-adrenoceptor antagonist, were investigated in rat models of fluid transport and transit in vivo. Fluid transport was investigated in the pentobarbitone-anaesthetised rat using a gravimetric technique (Young and Levin, 1990). Colonic transit was assessed in the conscious rat by following the transit of a ^{51}Cr label administered into the proximal colon via a chronic indwelling cannula implanted three days before the experiment. This model utilises the geometric centre method to quantify transit. Data is given as mean ± SEM (n) and statistical analysis carried out by Student's t-test.

Under basal conditions the rat colon displayed a large fluid absorption, (-704 ± 31 µg fluid $cm^{-1}min^{-1}$ (10)). Administration of idazoxan (1 μgkg^{-1} to 10 $mgkg^{-1}$ i.p.) caused an apparent reversal of this basal fluid absorption to that of secretion in a dose-dependent manner. The lowest effective dose was 10 μgkg^{-1} i.p. and fluid absorption was effectively abolished at 500 μgkg^{-1} i.p. (-17 ± 20 µg fluid $cm^{-1}min^{-1}$ (10)) and reached maximal secretion at 1 $mgkg^{-1}$ i.p. (+399 ± 16 µg fluid $cm^{-1}min^{-1}$ (9)). Use of the α_2-agonist UK-14,304 (10 $ngkg^{-1}$-1 $mgkg^{-1}$ i.p.) caused large increases in fluid absorption, the maximal effective dose being 100 μgkg^{-1} i.p. (-1097 ± 25 (10)). These effects could be abolished by idazoxan (1-10 μgkg^{-1} i.p.), these doses having no effect by themselves.

Basal transit in the rat colon was 0.36 ± 0.02 (14). Idazoxan 3-30 $mgkg^{-1}$ i.p. caused a dose-dependent, but non-significant acceleration of transit (5-30%). UK-14,304 (100 $ngkg^{-1}$-1 $mgkg^{-1}$ i.p.) caused a dose-dependent inhibition of transit. The inhibition observed by 10 μgkg^{-1} UK-14,304 (0.22 ± 0.04 (6)) was totally reversed by idazoxan (3 $mgkg^{-1}$) to 0.43 ± 0.05 (6), a dose which on its own had no effect on transit.

These results suggest a tonic role for α-adrenoceptors in the regulation of fluid transport in the rat colon by enhancing fluid absorption and blockade of these receptors results in secretion. Maximal fluid secretion with idazoxan is observed at doses which have no effect on transit. The inhibition of transit with UK-14,304 is accompanied with large increases in fluid absorption. These results would suggest that in the colon excessive fluid secretion on its own is not a prerequisite for the stimulation of propulsion.

Young, A. and Levin, R.J. (1990). Gut, 31, 43-53.

480P [4-Cl-D-Phe^6, Leu^{17}] VIP IS NOT AN EFFECTIVE VIP ANTAGONIST IN PREPARATIONS OF RAT COLONIC MUCOSA

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Pandol et al (1986) have shown [4-Cl-D-Phe^6, Leu^{17}] VIP to antagonise short circuit current (s.c.c.) responses of cultured human colonic mucosal cells to vasoactive intestinal peptide (VIP). The purpose of the present investigation was to obtain evidence for a neurosecretory role for VIP in the rat colon using this compound. Muscle stripped sheets of rat colonic mucosa were set up in Ussing chambers to detect changes in net electrogenic ion transport. Values of s.c.c. quoted are for an exposed membrane area of $0.64cm^2$.

After 60min, basal s.c.c. was 15.6 ± 1.2 µA (n=57). Cumulative application of acetylcholine (ACh, 1.4-385 µM) bethanechol (BCh, 0.005-6.2mM) and VIP (0.006-0.77µM) gave concentration dependent increases in s.c.c. Ed_{50} values were 9.9 ± 3.6µM for ACh (n=4) 157 ± 28µM for BCh (n=4) and 42 ± 6nM for VIP (n=16). Maximum responses were 160 ± 14µA for ACh, 157 ± 12µA for BCh and 105 ± 12µA for VIP. The responses were sided i.e. the preparation was virtually insensitive to ACh (5.5µM) and VIP (0.03µM) when the agents were applied to the mucosal side. Electrical field stimulation (EFS, 10Hz 1ms for 15s) of mucosal preparations gave voltage dependent increases in s.c.c. ranging from 11 ± 5µA at 2.5v (0.5 mA, n=4) to 79 ± 8µA at 40v (33mA, n=11). Hyoscine (5.2 ,10.4µM) reduced the responses to EFS (20 or 40v) by 23 ± 4% ($P<0.05$, n=7) whereas TTX (3.1µM) reduced responses by 95 ± 3% ($P<0.01$, n=5). Basal s.c.c. levels were unaffected by hyoscine ($P>0.05$, n=7) but reduced by 27 ± 5% by TTX ($P<0.05$, n=14).

Cumulative dose-response curves to VIP were displaced to the left by [4-Cl-D-Phe^6, Leu^{17}] VIP (0.3µM, n=3; 3µM, n=3; 12µM, n=2). This may have been a reflection of comparable increases in tissue sensitivity to VIP as seen in control experiments. (n=4) Some reduction of the response to VIP (0.38µM) was observed with the highest concentration of antagonist (12µM) but this was almost certainly due to application of VIP against a raised basal s.c.c. caused by the antagonist. [4-Cl-D-Phe^6, Leu^{17}] VIP caused an increase in basal s.c.c. ranging from 5µA (0.3µM) to 38µA (12µM).

The ineffectiveness of [4-Cl-D-Phe^6, Leu^{17}] VIP may be due to the slightly lower concentrations used in this study. Pandol et al (1986) recommended 30µM. Alternatively species differences i.e.human versus rat; or preparation differences i.e.cultured epithelial cells versus muscle stripped mucosal sheets, may have been the explanation.

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481P THE EFFECT OF NPY AND SOME NPY C-TERMINAL FRAGMENTS ON THE FIELD STIMULATED RAT VAS DEFERENS

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It has been proposed that at least two NPY receptor subtypes exist (post synaptic NPY-Y1 and pre-synaptic NPY-Y2 receptors, Wahlstedt et al.,1986). The purpose of the present study was to characterise the putative presynaptic NPY-Y2 receptors in the rat vas deferens preparation using a range of porcine NPY C-terminal fragments.

Prostatic portions of rat vasa deferentia were suspended in Krebs' bicarbonate buffer at 37°C and bubbled with $95\%O_2/5\%CO_2$. Contractions were elicited by passing pulses (10ms duration, 0.035Hz, 15v) between parallel platinum electrodes placed either side of the tissue. Twitch responses were recorded isometrically under an initial tension of 1g.

NPY produced a concentration related inhibition of the twitch response of the rat vas deferens with a mean (±S.E.M) IC50 value of 0.2 ± 0.08μM. Similarly NPY_{18-36} and $NPY_{1-4Eaca25-36}$ also inhibited the electrically evoked twitch response. Thus, NPY_{18-36} inhibited the twitch response with a mean (±S.E.M) IC_{50} value of 4.7 ± 2.5μM. The inhibition produced by $NPY_{1-4Eaca25-36}$ however, failed to reach an IC_{50} level (mean ± S.E.M inhibition at 3μM was 30.6 ± 4.3%). In contrast, NPY_{13-36} produced a transient enhancement of the response to field stimulation (mean ± S.E.M peak enhancement produced by NPY_{13-36} was 161 ± 26% at 10μM). NPY_{13-36}, at a concentration of 3μM, failed to antagonise the inhibitory activity of NPY (mean ± S.E.M NPY IC_{50} values were 0.2 ± 0.07μM and 0.18 ± 0.01μM in the absence and presence of NPY_{13-36} respectively). Furthermore, preincubation with the α_2 antagonist Wy26392 (3μM) failed to inhibit NPY_{13-26} induced enhancement (mean ± SEM peak enhancement 114.2 ± 17.3% and 90.8 ± 28.4% in the absence and presence of Wy26392 respectively).

In conclusion, the present study supports the findings of other workers that NPY - like agonist activity is retained in NPY_{18-36} and $NPY_{1-4Eaca25-36}$. The transient enhancement of the twitch response by NPY_{13-36} however, is unexpected and differs from the findings of other workers (Danho et al., 1988). This anomaly could possibly be due to differences in stimulation perameters or alternatively that the NPY_{13-36} used in this study differs from that used by others. Finally, the mechanism by which NPY_{13-36} enhances the response to field stimulation is not clear but it seems it is not due to NPY or α_2 receptor antagonism.

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482P DIFFERENTIAL INVOLVEMENT OF κ OPIOID RECEPTORS IN REGULATING FLUID TRANSPORT IN THE RAT SMALL AND LARGE INTESTINE *IN VIVO*

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Although evidence exists regarding the effects of μ-receptors in controlling intestinal fluid transport, there is little evidence for the role of k-receptors. Recent studies have demonstrated the importance of k-receptors in prevention of castor-oil induced diarrhoea in the mouse via an action on fluid transport, rather than motility (Shook et al., 1989). The effects of the k-agonists ethylketocyclazine (EKC), U50488H and U69593 on fluid transport in the pentobarbitone anaesthetised (60 $mgkg^{-1}$ i.p.) rat jejunum and colon were determined using a previously published technique (Young and Levin, 1990). Dose-response relationships for the k-agonists were determined over the range 100 $ngkg^{-1}$ i.p. to 5 $mgkg^{-1}$ i.p..

In the two areas investigated these compounds increased basal fluid absorption with an apparent order of potency of U69593 > U50488H > EKC. The lowest effective doses (LED), maximal effective doses (MED) and associated fluid changes are shown in Table 1. As can be seen these compounds had by far the greatest effect in the colon.

Table 1 - The effects of EKC, U50488H and U69593 on increases in fluid absorption above basal levels in the rat jejunum and colon given as μg fluid cm^{-1} min^{-1}. Lowest (LED) and maximal effective doses (MED) are given on a kg^{-1} i.p. basis. Data is in the form of mean ± SEM (n).

		Jejunum		Colon	
EKC	LED	500 μg	43±12 (9)*	100 μg	73±21(11)*
	MED	5 mg	119±47 (6)**	1 mg	223±41 (6)***
U50488H	LED	100 μg	75±24(11)*	10 μg	104±43(11)**
	MED	5 mg	160±37(12)**	500 μg	399±61 (9)***
U69593	LED	10 μg	104±20(12)**	1 μg	111±36(10)***
	MED	1000 μg	217±37 (9)***	100 μg	542±37(10)***

Compared to basal values *p<0.05, **p<0.01 and ***p<0.001 using Student's t-test.

The effects in Table 1 could be blocked using the selective k- antagonist norbinaltorphimine at 1-10 μg/kg i.p.. Thus, k-receptors have a significant role to play in the regulation of intestinal fluid transport in the rat, particularly in the large intestine.

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483P DEMONSTRATION OF 5-HT_{1A} AGONIST ACTIONS OF 5-CARBOXAMIDOTRYPTAMINE IN THE ISOLATED TRANSMURALLY STIMULATED ILEUM OF THE GUINEA-PIG

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Fozard & Kilbinger (1985) described the 5-HT_{1A} receptor mediated inhibition of the transmurally stimulated ileum of the guinea-pig. 8-OH DPAT produces a biphasic concentration-effect curve in this preparation, only the 1st phase being blocked by 5-HT_{1A} antagonists. 5-carboxamidotryptamine (5-CT) demonstrates 5-HT_{1A} agonism but also has smooth muscle relaxant effects (Feniuk et al., 1983) which are blocked by mesulergine (Kalkman et al., 1986). We have studied the possibility of isolating the 5-HT_{1A} receptor mediated actions of 5-CT in the transmurally stimulated preparation.

Segments of the terminal ileum from male guinea-pigs (300-600g) were set up in modified Krebs solution (mM:NaCl 118.4; KCl 4.8; $NaHCO_3$ 25.0; $MgSO_4$ 0.6; KH_2PO_4 1.2; glucose 11.2; $CaCl_2$ 1.25) gassed with 5% CO_2 in oxygen and maintained at 35°C. For smooth muscle relaxation experiments histamine-precontracted preparations were used (Feniuk et al., 1984). In stimulation experiments segments were stimulated transmurally (1.0 msec, 0.05H_z, 17-20mA). All tissue responses were measured isotonically.

In histamine-precontracted preparations 5-CT and 8-OH DPAT produced concentration-related relaxations (EC_{50} = 0.0143 and 3.0μM respectively). Spiperone (0.3μM) or mesulergine (3μM) antagonised 5-CT responses (pA_2 = 7.6 and 7.5). The mechanism of the 8-OH DPAT response was different as it was not affected by spiperone (0.3 μM).

In transmurally stimulated tissues mesulergine (3μM) did not shift the concentration-effect curves to 8-OH DPAT or 5-CT. However, the maximum inhibition by 5-CT was reduced from 70% to 47% of the predose response. The 5-HT_{1A} antagonist BMY 7378 (Yocca et al., 1987) antagonised the inhibitory action of 5-CT in stimulated preparations in the presence of mesulergine (3μM) with a pA_2 value of 8.0(7.8-8.4) and a Schild plot slope of 1..0 (0.7-1.2).

In conclusion, 5-CT in the presence of mesulergine, may be a satisfactory agonist for the evaluation of antagonists at functional 5-HT_{1A} receptors in the isolated, transmurally stimulated ileum of the guinea-pig.

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484P DIFFERENCES IN THE EFFECTS OF 5-HT_3 RECEPTOR ANTAGONISTS AGAINST THE 5-HT_3 MEDIATED RESPONSES TO 5-HT AND 2-Me 5-HT IN THE GUINEA-PIG ILEUM LONGITUDINAL MUSCLE

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Butler et al. (1988) have shown that GR38032F (GR) antagonises the contractile response to 2-methyl 5-hydroxytryptamine (2-Me 5-HT) with a similar pA_2 value to that obtained against the second (low affinity) phase of the response to 5-HT in the isolated guinea-pig ileum longitudinal muscle strip (GPI). It has been shown that 2-Me 5-HT is a selective agonist for 5-HT_3 receptors (Richardson et al., 1985) and 2-Me 5-HT has recently been used to differentiate the 5-HT_3 and proposed 5-HT_4 receptor-mediated responses in the guinea-pig ileum (Craig & Clarke, 1990).We have compared the actions of three 5-HT_3 receptor antagonists against the responses to 5-HT and 2-Me 5-HT in the GPI with a view to confirming the identity of these agonist mechanisms.

The method used for the GPI was that of Buchheit et al.(1985). When 5-HT was used as the agonist the Krebs' solution contained methysergide (1 μM). Non-cumulative concentration-response curves to 5-HT (1 nM-1 mM) and 2-Me 5-HT (0.3 μM- 10 mM) were constructed using a 10-15 min dose cycle. Antagonists were equilibrated for 60 mins.

Analysis of 5-HT and 2-Me 5-HT concentration response curves.

Antagonist	Agonist	pA_2	slope	control max.(%)	% max. at 1 μM antagonist
GR38032F	5-HT	6.9 (6.7-7.9)	0.98 (0.33-1.63)	112 (108-117)	135 (130-139)*
	2-Me 5-HT	7.0 (6.9-7.2)	1.07 (0.84-1.29)	96 (93-100)	110 (103-119)*
Zacopride	5-HT	8.1 (7.8-8.7)	1.07 (0.76-1.38)	98 (91-104)	91 (86-98)
	2-Me 5-HT	7.7 (7.5-8.0)	1.37 (1.07-1.67)	89 (87-92)	70 (64-78)*
ICS205930	5-HT	7.1 (6.9-7.3)	2.94 (2.14-3.74)	104 (102-106)	241 (208-276)*
	2-Me 5-HT	7.9 (7.6-8.1)	0.85 (0.65-1.05)	102 (94-111)	38 (26-49)*

95% confidence limits shown in brackets. Four concentrations (over a 10 fold range) of each antagonist were tested.
* - Significantly different from control ($p<0.05$). n=4.

ED_{50} values were 2.4 (2.1-2.7)μM for 5-HT and 7.2 (6.6-8.1)μM for 2-Me 5-HT. The pA_2 values obtained with GR and zacopride were independent of the agonist used, consistent with 5-HT and 2-Me 5-HT acting at the same receptor type, however, this was not the case with ICS 205930. Also, GR was a surmountable antagonist of both 5-HT and 2-Me 5-HT whereas both zacopride and ICS 205930 were surmountable only against 5-HT, maximum responses to 2-Me 5-HT being depressed .The reason for the insurmountable antagonism observed with 2-Me 5-HT but not with 5-HT is unknown. The possibilities that zacopride and ICS 205930 may be slowly displaced from receptors and that the receptor reserve for 2-Me 5-HT is small in this tissue should be considered.

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485P INVESTIGATION OF THE 5-HT RECEPTOR MECHANISM MEDIATING RELAXATION OF GUINEA-PIG ASCENDING COLON

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5-HT induces relaxation of guinea-pig isolated ascending colon (GPAC) through a mechanism which is neuronally mediated and blocked by tetrodotoxin (TTX) (Costa and Furness, 1979; Kojima and Shimo, 1986). The purpose of the present study was to further characterise the 5-HT receptor mediating this relaxation.

The muscle layers of GPAC were dissected from the attached mucosa and set up in the longitudinal plane under a resting tension of 0.5-1.0g. Tissues were bathed in Krebs-Henseleit solution containing GR38032F (10μM) and ketanserin (1μM) at 34°C and gassed with 95% O_2/5% CO_2. The tissues were contracted with a submaximal concentration of carbachol (0.3μM), and when a stable contraction was obtained, a single dose of 5-HT (or other 5-HT agonist) was added. When the maximum response had been achieved the tissue was washed out. Two sequential concentration-response curves were constructed and where appropriate antagonists were equilibrated for 30 mins before construction of the second curve. Mean values are n=4 throughout.

5-Hydroxytryptamine (5-HT) produced a monophasic concentration-response curve with an EC_{50} value of 11.1 (8.4-14.6, 95% confidence limits)μM. 5-carboxamidotryptamine (5-CT) was a full agonist with an equipotent molar ratio of 0.1 (0.03-0.4) (where 5-HT=1). α-Methyl-5-HT (1-300μM) was a partial agonist producing 36.0±3.9% of the maximum response to 5-HT. 2-Methyl-5-HT (up to 100μM) was without effect. The responses to 5-HT and 5-CT were antagonised by TTX (0.3μM). GR43175 (1-300μM) produced large relaxations but these were not affected by TTX. Methysergide (0.1μM) and metergoline (0.1μM) produced parallel rightward shifts of the 5-HT concentration response curve from which pK_B values of 8.0±0.3 and 7.3±0.1 respectively were calculated. Higher concentrations of methysergide (1-10μM) and metergoline (1μM) caused a a further rightward shift which was unsurmountable. Neither methysergide (1μM) nor metergoline (1μM) affected relaxations to isoprenaline (0.003-1μM) and therefore appeared to be selective for 5-HT. Spiperone (0.3μM) had little effect on the 5-HT response, reducing the maximum response by 20.0±9.3% with a concentration ratio of only 2.2 (0.6-7.6).

The antagonist activity of methysergide differs from work by Costa and Furness (1979) and Kojima and Shimo (1986) where methysergide was inactive. The relaxation in the present study is unlikely to involve a $5\text{-}HT_{1A}$ receptor due to the low activity of spiperone. Also the response is not mediated by the subtype of $5\text{-}HT_1$-like receptor at which GR43175 is an agonist. The present results suggest that 5-HT produces neuronally mediated relaxations via a $5\text{-}HT_1$-like receptor but its full identity remains to be established.

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486P COMPARISON OF THE EFFECTS OF DELTA-AMINOLAEVULINIC ACID AND MUSCIMOL IN RAT JEJUNAL PREPARATIONS

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It has been suspected for many years that delta-aminolaevulinic acid (ALA), formed during the biosynthesis of haem, may play a role in the pathogenesis of acute porphyric attacks. Its concentrations in blood during these attacks are raised many-fold from normal values of 24-270 nmol/l to 9-12 μM/l (Gorchein & Webber, 1987). At 1 μM concentrations in vitro, ALA can inhibit potassium-stimulated release of GABA from nerve endings (Brennan & Cantrill, 1979). The present experiments compare responses to ALA (0.01 μM - 3.0 mM) with those of the $GABA_A$ agonist, muscimol, and assess the effects of pretreatment with $GABA_A$ antagonists in isolated preparations of rat jejunum.

The preparations of jejunum were bathed in oxygenated Krebs solution at 37 °C. Contractions of the preparations were recorded by an isotonic transducer and displayed on a calibrated Washington 400 MD2R oscillograph. Both muscimol (0.03 μM - 0.3 mM) and ALA (1.0 μM - 3.0 mM) increased the amplitude of contractions ($P < 0.05$). Resting length of the preparation was reduced by muscimol (1.0 μM - 0.3 mM) and by ALA (0.1 μM - 3.0 mM) ($P < 0.05$).

Effects of all concentrations of muscimol were blocked by bicuculline (10 μM). However, effects of ALA were significantly attenuated by bicuculline ($P < 0.05$) only within the concentration range of 0.1 - 1.0 mM ALA. At concentrations of 0.01 - 0.05 μM ALA, bicuculline significantly enhanced ($P < 0.05$) responsiveness of the preparation to ALA, increasing contraction amplitude and decreasing resting length. Picrotoxin (10μM) likewise increased responses of the preparation to concentrations of 0.01 - 0.05μM ALA ($P < 0.05$).

In rat jejunum, $GABA_A$ receptors mediate cholinergic contraction along with a non-adrenergic non-cholinergic relaxation (Krantis & Harding, 1987). The present results suggest that ALA at higher concentrations may act as a partial agonist at $GABA_A$ receptors, while at the lower concentrations, comparable with those occurring in acute porphyric attacks, it has significant but complex effects on GABA-mediated functions. This may arise from effects at GABA autoreceptors, as proposed by Brennan & Cantrill (1979), and many of these are baclofen-sensitive (Giralt et al., 1990). The observed paradoxical effects of $GABA_A$ antagonists may be due to an interaction between $GABA_A$ and $GABA_B$ receptors, as suggested by Allan & Dickinson (1986) who found bicuculline to reverse antagonism of the baclofen response.

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487P THE RESPONSE OF THE HUMAN UTERUS *IN VITRO* TO LEUKOTRIENES

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The human myometrium has been shown to be capable of leukotriene (LT) biosynthesis. Their presence may be indicative of them being mediators in normal physiological processes, for example, implantation. Alternatively, they may be synthesised as a result of a pathological disorder such as dysmenorrhoea. In this study the effect of LTB_4, C_4 and D_4 on isolated human myometrium was examined.

Samples of human myometrium were obtained from pre-menopausal patients at hysterectomy or from pregnant patients during Caesarean section. The myometrial strips were set up as previously described (Massele & Senior, 1981) and were superfused with Krebs solution (37°C, 95% O_2/5% CO_2) at 2 ml min^{-1}. LT was administered as a bolus dose into the superfusate, only one dose per tissue was used because of a decrease in sensitivity of the tissue to the compounds.

It was found that tissue obtained from non-pregnant donors in the luteal phase of the menstrual cycle was the most sensitive to LT, this confirms earlier findings in this laboratory (Clayton et al, 1985). LTB_4 (10^{-10}-10^{-7} moles) had no discernible effect on the tissue. LTC_4 and D_4 (10^{-10}-10^{-8} moles) evoked a qualitatively similar response, the former producing the most pronounced effect, the response being predominantly one of inhibition of spontaneous activity which was frequently preceded by a dose-independent contraction. The inclusion of indomethacin (2.79 x 10^{-6}M) in the perfusate had little effect on the response. The LT antagonist, ICI 198615 (Krell *et al.*, 1985) (10^{-6}M), abolished the response to LTC_4. Tissue obtained from pregnant donors was the least responsive to the LTs. On tissue which exhibited marked spontaneous activity (the patient had reached full term) only a contractile response was obtained.

In conclusion it would seem that the human myometrium *in vitro* is not particularly sensitive to exogenous LT (up to 50% of non-pregnant donors and 70% of pregnant donors did not respond at all). The initial work with indomethacin suggests that the response is direct and not mediated via the cyclo-oxygenase pathway. The responsiveness of the tissue was also apparently dependent on the hormonal status of the donor. This may be important when extrapolating to the *in vivo* situation as well as the fact that preliminary studies in this laboratory seem to indicate that LTC_4 and to some extent LTD_4 cause a contraction of isolated human uterine artery.

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488P UTERINE TOLERANCE TO RELAXANTS *IN VIVO*: LACK OF CROSS TOLERANCE BETWEEN RELAXIN, SALBUTAMOL AND CROMAKALIM

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Tolerance to the relaxant effects of relaxin, salbutamol (a β-adrenoceptor agonist) and cromakalim (a potassium-channel opener) occurs in the uterus of the rat in vivo (Downing & Hollingsworth, 1989; Abel & Hollingsworth 1986, Downing et al., 1989). Relaxin may modulate intracellular cAMP concentrations and open K-channels (Downing & Hollingsworth, 1990). Relaxants with similar mechanisms of action may exhibit cross-tolerance. The aim of this study was to investigate cross-tolerance between relaxin, salbutamol and cromakalim, and between cromakalim and RP 49356 (also a K-channel opener, Weston 1988).

Female rats, 200-250g, were anaesthetized with tribromoethanol, 240mg kg-1, bilaterally ovariectomized and given an intra-uterine balloon and jugular vein cannula. After 24h, uterine responses to relaxin (2-20μg kg-1), salbutamol (10-200μg kg-1), cromakalim (0.05-0.1mg kg-1) or RP49356 (0.05-0.25mg kg-1) iv, recorded for 60 min were expressed as % inhibition of integral of uterine contractions for the 60 min before each dose. Tolerance was induced by iv infusion (relaxin; 20μg kg-1 hr-1 for 40h, salbutamol; 120μg kg-1 hr-1 for 24h) or injection (cromakalim; 3 x 1mg kg-1, 8h apart). Vehicles were saline, 0.15ml hr-1 or 14% ethanol/saline, 3 x 1ml kg-1. After tolerance induction dose response curves to the relaxants were constructed. Pre- and post-tolerance log ID50 values were determined for each rat by probit analysis.

Uterine sensitivity to relaxin was unchanged in vehicle, salbutamol- or cromakalim-treated rats (log ID50s (μg kg-1): pre-salbutamol, 0.85±0.07, post-salbutamol, 1.03±0.08; pre-cromakalim, 0.77±0.08, post-cromakalim, 0.64±0.06). Sensitivity to salbutamol or cromakalim increased in both saline and relaxin-infused rats ($p<0.05$) (log ID50s salbutamol: (μg kg-1) pre-relaxin, 1.83±0.22, post-relaxin, 1.16±0.13; cromakalim: (mg kg-1) pre-relaxin, -1.12±0.15, post-relaxin, -2.21±0.34). Sensitivity to RP49356 increased 4-fold in vehicle ($p<0.005$) but decreased 2-fold in cromakalim treated rats (log ID50s: (mg kg-1) pre-vehicle, -0.95±0.08, post-vehicle, -1.59±0.08; pre-cromakalim, -0.95±0.06, post-cromakalim, -0.72±0.11).

In conclusion, no cross tolerance was observed between relaxin, salbutamol and cromakalim, suggesting that the site of tolerance development differs between the three relaxants despite possible similarities in mechanism of action. Cross tolerance was observed between two K-channel openers, cromakalim and RP49356.

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489P THE ACTIONS OF SALMETEROL AND TWO NON-SELECTIVE β-ADRENOCEPTOR AGONISTS, ISOXSUPRINE AND NYLIDRIN, ON β_1- AND β_2-ADRENOCEPTORS

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Salmeterol, a selective β_2-adrenoceptor agonist, was described by Bradshaw et al. (1987) as a long-lasting alternative to salbutamol on the basis of its persistence in the vicinity of its receptor. It was suggested that this was due to its increased binding to an exoreceptor (Brittain et al., 1981; Bradshaw et al., 1987; Nials & Coleman, 1988).

It had previously been noted by one of us that similar long-acting properties were shared on the guinea-pig isolated tracheal muscle by two other long-chain, but non-selective, β-adrenoceptor agonists, isoxsuprine and nylidrin. All three agonists were tested on isolated tissues which contained a predominance of β_2-adrenoceptors (guinea-pig trachea, rat uterus and bovine sphincter pupillae) and on those which contained a predominance of β_1-adrenoceptors (guinea-pig ileum and perfused guinea-pig heart). In each tissue with β_2-adrenoceptors, after the establishment of the action of each of the three agonists, the addition of sotalol reversed the action of the agonist. After washout the action of the agonist was re-established. Application of sotalol again reversed the action of the agonist. Washout again re-established the agonist response. This was repeated up to four times (over two hours), but none of the tissues showed more than 40 per cent recovery from the effect of the agonist. With the guinea-pig trachea, after three washout periods, the re-establishment of the relaxant action for salmeterol was 80.2% (s.e.mean 4.8; n=4), for isoxsuprine 61.0% (s.e.mean 9.6; n=4) and for nylidrin 71.1%(s.e.mean 15.6; n=3).

However, in the tissues with β_1-adrenoceptors a repetition of this experimental protocol resulted in the washout of the agonist after the first reversal with sotalol. In all the tissues isoprenaline gave an agonist response which was antagonised by sotalol with complete recovery after one washout period. This suggests that the exoreceptor is unique to the β_2-adrenoceptor.

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490P EFFECTS OF A NOVEL PERFLUOROCHEMICAL EMULSION ON LIVER ENZYMES AND THE RESPONSE TO PENTOBARBITAL ANAESTHESIA IN RATS

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Perfluorochemical (PFC) emulsions have therapeutic value for respiratory gas transport (Lowe, 1988), but the consequences of their use on tissue metabolic functions have not been studied in detail. This is surprising since PFCs have been tested in animal studies as adjuncts to anti-cancer drugs (Teicher & Holden, 1987). We have therefore examined the effects of pre-treatment with a novel PFC emulsion (Sharma et al., 1987) on liver enzymes and the response to the pentobarbital-based anaesthetic, Equithesin, in rats.

Male Wistar rats (body weight (b.w.: 200-256g; n = 16) were injected intravenously via a tail vein with 10 ml/kg b.w. of either (i) a novel 20% (w/v) perfluorodecalin (FDC) emulsion containing 1% (w/v) of a C-16 oil additive, perfluoroperhydrofluoranthrene, and 4% (w/v) Pluronic F-68 (Sharma et al., 1987) or (ii) saline (0.9% w/v NaCl). 72 hr later, animals were injected intraperitoneally with 2 ml/kg b.w. of Equithesin and the "sleeping time" (ST) measured (Bousquet et al., 1965). After 24 hr, animals were killed and their livers removed and weighed. Liver cytochromes P-450 (P-450) concentration and aryl esterase (LAE) activity were determined as previously (Armstrong & Lowe, 1989, 1990).

Mean (± s.e.m.) P-450 concentration following injection of the novel emulsion (0.54 ± 0.10 nmol/mg protein; n = 8) was significantly ($P < 0.05$) greater than in controls (0.29 ± 0.03 nmol/mg protein; n = 8). In contrast, mean LAE activities in PFC emulsion-injected rats (1.12 ± 0.12 µmol/min/mg protein; n = 8) and controls (1.15 ± 0.15 µmol/min/mg protein; n = 8) were not significantly different. Mean ST in rats injected with the novel emulsion (20 ± 8 min; n = 8) was significantly lower ($P < 0.05$) than in controls (45 ± 7 min; n = 8).

These results show that FDC emulsion can induce liver P-450 in male rats in agreement with previous findings (Armstrong & Lowe, 1989). The decrease in ST following injection of the novel emulsion suggests that PFC-mediated liver P-450 induction can enhance pentobarbital metabolism and thus reduce its anaesthetic properties. This is supported by related studies showing increased antipyrine metabolism in rats transfused with the commercial PFC emulsion, Fluosol-DA 20% (Shrewsbury & White, 1988).

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491P TISSUE RETENTION OF PERFLUOROCHEMICALS IN RATS

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Marked alterations in lymphoid tissue weights can occur in rodents injected with emulsified perfluorochemicals (PFCs; Lowe, 1988). It is generally assumed that the principal recipients of such compounds in vivo are the liver and spleen (e.g. Lowe, 1988), although this has not been studied systematically. We have therefore assessed tissue uptake and retention of PFCs following injection of their emulsions in rats.

Male Wistar rats (body weight (b.w.): 150-300g; n = 48) were injected intraperitoneally under light ether anaesthesia with 30 ml/kg b.w. of either (i) Fluosol-DA 20% (F-DA; Green Cross, Japan), (ii) a novel 20% (w/v) perfluorodecalin (FDC) emulsion containing 1% (w/v) of a C-16 oil additive, perfluoroperhydrofluoranthrene (ISC Chemicals, Avonmouth) and 4% (w/v) Pluronic F-68 (Sharma et al., 1987), or (iii) saline (0.9% w/v NaCl). At 24 hr, 72 hr, 1, 2, 3 or 4 weeks after injection, animals were killed by cervical dislocation and their livers and spleens removed, weighed and frozen in liquid N_2. Tissue PFC concentrations were determined using a modification of the gas chromatographic method of Yamanouchi et al. (1975), following extraction with either carbon tetrachloride (BDH, Poole) or tetrachloroethylene (BDH). Samples (1 µl) were analysed using a Pye Unichem 4500 gas chromatograph.

Uptake of PFCs, as determined by measurement of total tissue PFC concentrations, was greater in spleen than liver, irrespective of emulsion injected. Maximal mean (± s.e.m.) tissue PFCs occurred 72 hr after injection of F-DA (spleen: 86.3 ± 1.3 mg/g; liver: 30.5 ± 1.4 mg/g), but were more variable in rats injected with the novel emulsion. At 4 weeks post-injection, spleen PFC concentrations had fallen to < 5% of maximum values and were undetectable in the liver. Marked differences in the time course of retention of individual PFCs occurred in liver and spleen. For example, in animals receiving the novel emulsion, mean spleen FDC and C-16 oil concentrations were maximal 24 hr after injection (FDC: 21.0 ± 1.5 mg/g; C-16: 0.6 ± 0.03 mg/g), whereas maximum liver concentrations (FDC: 4.2 ± 1.1 mg/g; C-16: 0.2 ± 0.06 mg/g) occurred 72 hr post-injection in both cases.

These results show both quantitative and qualitative differences between rat liver and spleen in their uptake and retention of PFCs. One explanation is the variation in molecular weight of individual compounds which is known to influence their tissue retention time (Yamanouchi et al., 1975).

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492P RADIOLIGAND BINDING TECHNOLOGY WHICH ELIMINATES THE REQUIREMENT FOR SEPARATION OF FREE AND BOUND RADIOLABEL

T.P. Wood, P. Towers, K. Swann, J.C. Ireson, N. Bosworth and M.G. Wyllie, Pfizer Central Research and Amersham International PLC.

Classically free and bound radiolabel are separated by filtration or centrifugation prior to quanitification by liquid scintillation spectrophotometry. In this communication we describe a novel competitive binding system for the characterisation of interactions at the epidermal growth factor (EGF) binding site; this scintillation proximity assay (SPA system does not require separation of components prior to counting.

In an aqueous environment, in order to emit light, relatively weak β-emitters (^{3}H and ^{125}I [Auger electrons]) must be in close proximity to scintillant molecules, otherwise the radioactive energy is dissipated by the medium. In the case of EGF, this proximity can be achieved by coupling EGF receptor protein to a fluomicrosphere (a support matrix consisting of a bead impregnated with fluor). Bound radiolabelled EGF is thus sufficiently close to ensure activation of the fluor and subsequent light emission.

The assay system involves incubating samples for 24 hours at room temperature in 20mM HEPES, pH7.5, containing 0.5% BSA. The incubation medium also contains a cocktail of an anti-mouse antibody covalently surface bonded to a latex fluomicrosphere, a mouse monoclonal antibody directed at a non-EGF binding region and a Triton X-100 solubilised A431 post nuclear supernatant cell membrane preparation.

Under these conditions binding is saturable (Kd 2nM) with over 90% of the binding being specific (defined as that displaced by 250nM unlabelled EGF). Results using SPA correlated well with those using a filtration separation assay. Binding is unaffected by a wide range of biological molecules active at other receptors (including peptide receptors).

The assay system described could be adapted for a wide range of other ligands, offering a new dimension in the quantification of receptor-ligand interactions.

493P *IN VITRO* METABOLISM OF PHYSOSTIGMINE BY HUMAN PLASMA: INTERACTIONS WITH OTHER DRUGS

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In vitro loss of physostigmine from samples of human plasma is enzyme catalysed. Using an initial concentration of 100 ng ml^{-1}, Km and Vmax were 13 ng ml^{-1} and 0.45 ng ml^{-1} min^{-1}, respectively. Below 10 ng ml^{-1} the kinetics were first order (Hurst & Whelpton, 1989). High concentrations of neostigmine have been used to stabilise samples before assay (Whelpton & Moore, 1985). This study investigated inhibition of *in vitro* hydrolysis of physostigmine with regard to a) total inhibition prior to analysis and b) potential interaction with drugs thought to be metabolised by plasma esterases.

[^{3}H]-Physostigmine (10 ng ml^{-1}) was incubated in blood bank plasma at 37°C. Inhibitors were pre-incubated for 15 min prior to addition of the drug. Periodically, samples were withdrawn and extracted with toluene to separate physostigmine from radioactive metabolites. The apparent first order rate constants (k) were obtained by iteratively fitting the concentration-time curves to a single exponential model (Neilson-Kudsk, 1983).

Neostigmine (3.3 µM) pyridostigmine (3.8 µM) and soman (0.25 µM) completely inhibited physostigmine metabolism for the duration of the experiment (10 h). Mercury(II) and copper(II) ions (400 µM) had no effect but high concentrations of fluoride (2 mM) increased the half-time (± s.d.) from 20 ± 1 to 34 ± 2 min. Aspirin (560 µM) procaine (37 µM) and suxamethonium (25 µM) had no significant effect at the highest concentrations tested. Cocaine (29 µM) reduced the rate constant from 0.064 ± 0.002 min^{-1}, n=5 to 0.040 ± 0.001 min^{-1}, $P<0.001$. No effect was observed at 2.9 µM. Dibucaine (26 µM) reduced k from 0.069 ± 0.003, n=5 to 0.023 ± 0.001 min^{-1}, n=6, $P<0.001$. At 2.6 µM dibucaine, k was 0.063 ± 0.001 min^{-1}, $0.05>P<0.1$.

Considering that *in vitro* half-times are typically between 10 and 20 min, and that whole body clearances in human volunteers exceed liver blood flow (Whelpton, unpublished) plasma hydrolysis is likely to be an important metabolic step in the elimination of physostigmine *in vivo*. Competition for plasma esterases may be the basis of drug-drug interactions *in vivo* and such interactions may be predictable from *in vitro* studies.

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303P **Boyle SJ*, Meecham KG, Hunter JC & Hughes J**
Characterisation of [^{3}H]-CI-977 labelled κ opioid binding sites in rodent CNS

304P **Knight AR*, Gillard J & Wong EHF**
Characterization of the pharmacology of sigma opioid sites in guinea-pig brain and NCB20 cell membranes

305P **Sharp T*, Bramwell SR & Grahame-Smith DG**
Short-term lithium increases the release of hippocampal 5-HT evoked by electrical stimulation of the dorsal raphé nucleus in the rat *in vivo*

306P **Garratt JC*, Mason R & Marsden CA**
Electrophysiological study on the effects of DOI on dorsal raphé 5-HT neuronal firing

307P **Galzin AM*, Poncet V & Langer SZ**
5-HT_3 receptor agonists enhance the electrically-evoked release of [^{3}H]-5-HT in guinea-pig frontal cortex slices

308P **Crick H* & Wallis DI**
Effect of 5-HT on reflex responses of neonate rat spinal motoneurones

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Scopolamine-induced deficits in a primate object discrimination task are reversed by ondansetron (GR38032F)

310P **Hutson PH, Semark JE* & Middlemiss DN**
The TRH analogue MK-771 increases rat hippocampal but not striatal acetylcholine release *in vivo*

311P **Lambert DG* & Nahorski SR**
Inhibitory effects of phorbol esters on muscarinic receptor-linked inositol phosphate accumulation and intracellular Ca^{2+} homeostasis in SH-SY5Y human neuroblastoma cells

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Effects of chronic administration of a TRH analogue (RX77368) and atropine on cognitive behaviour and brain biochemistry in the rat

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Nicotinic receptors in the cerebral cortex in Alzheimer's disease and Parkinson's disease

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Drug manipulation reveals different pathways for the induction of purposeless chewing and facial tremor in rats

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Quisqualate stimulates phosphoinositide metabolism by interaction with more than one receptor mechanism

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Characterization of [^{3}H]-ifenprodil binding to the rat cerebral cortex

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Simultaneous intracellular recording from neurones of origin and termination of the perforant path during Mg^{2+}-free induced epileptogenesis *in vitro*

319P **Kaumann AJ*, Sanders L, Brown AM, Murray KJ & Brown MJ**
Human atrial 5-HT receptors: similarity to rodent neuronal 5-HT_4 receptors

320P **MacKenzie I*, Waterfall JF, Jones PS & Dunsdon RM**
Comparison of Class III antiarrhythmics and glibenclamide on action potentials from guinea-pig papillary muscle under normoxic and hypoxic conditions

321P **Armah BI*, Stenzel W, Raap A, Brückner R & Muster D**
BDF 9148: a cAMP independent positive inotropic agent that activates sodium channels without prolonging Q-T duration

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Antagonism of adenosine P_1 and muscarinic receptor activation of ^{86}rubidium efflux from guinea-pig left atria

323P **Robinson SC*, Wormald AD, Munsey TS, Bowmer CJ & Yates MS**
Myocardial energy production in rats with acute renal failure

324P **Holbrook M*, Shahid M & Coker SJ**
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Effects of glyceryl trinitrate and cultured vascular smooth muscle cells on platelet aggregation

327P **El Sayed AA, El Nahas AM, Towers JD & Haylor J***
Effect of nitro-arginine methyl ester on renal function in the isolated perfused rat kidney

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EDRF and vascular responsiveness in the perfused kidney of the spontaneously hypertensive rat

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Comparison of the regional haemodynamic profiles of Ro 31-6930, cromakalim and nifedipine using pulsed Doppler flowmetry in the anaesthetised normotensive rat

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Does progesterone-evoked suppression of human intracranial artery contractility involve potassium channel activation?

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Comparison of cromakalim-, pinacidil- and nicorandil-induced relaxation of rat isolated basilar artery

332P **Micheli D, Ratti E, Toson G, Trist D & Gaviraghi G**
Pharmacological profile of taludipine (GR53992B), a new long-acting calcium entry blocker

333P **Salvemini D*, Masini E, Mannaioni PF & Vane J**
Formation of nitric oxide-like activity from L-arginine in rat mast cells

334P **Purcell WM* & Hanahoe THP**
Human placenta: a novel source of mast cells

335P **Scotcher KP*, Jenner P, Irwin I, DeLanney LE, Langston JW & Di Monte A**
MPTP-induced ATP depletion in synaptosomes is inhibited by selegiline but not mazindol

336P **Loeschmann P-A*, Wachtell H, Jenner P & Marsden CD**
Behavioural effects of rolipram in MPTP-treated common marmosets

337P **Hadley AJ*, Flack JD & Buckingham JC**
Modulation of corticotrophin (ACTH) release *in vitro* by methylxanthines and adenosine analogues

338P **Cover PO*, Laycock JF, Gartside IB & Buckingham JC**
Evidence that vasopressin contributes to the stress-induced suppression of gonadotrophin release

339P **Baldwin HA*, Rassnick SR, Koob GF & Britton KT**
Ethanol withdrawal: a role for corticotrophin-releasing factor (CRF)

340P **File SE, Zharkovsky A* & Gulati K**
Baclofen reverses the signs of ethanol withdrawal in rats

341P **O'Connor SE*, Wood BE & Leff P**
Relative agonist potencies of methylene substituted ATP analogues for P_{2X}-purinoceptors in the rabbit ear artery

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Suramin is a slowly equilibrating but competitive antagonist at P_{2X}-purinoceptors in the rabbit ear artery

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P_2 purinoceptor-linked stimulation of labelled phosphatidic acid production in bovine aorta endothelial cells.

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Characterization of guanine nucleotide-sensitive bradykinin (BK) binding sites in bovine aortic endothelial cells

345P **Hudson AL* & Nutt DJ**
In vitro autoradiography of [^{3}H]-RX821002 in the rat CNS, a new ligand for identifying α_2-adrenoceptors

346P **Payvandi N*, Elliott JM & Heal DJ**
Radioligand binding to presynaptic α_2-adrenoceptors in rat cortex revealed by short-term DSP-4 lesioning

347P **Elliott PJ*, Mason GS, Stephens-Smith M & Hagan RM**
Use of a rotational model to demonstrate the existence of neurokinin NK-2 receptors in the rat substantia nigra

348P **Barnes S*, Hunter JC, Woodruff GN & Hughes J**
CCK_B receptor activation causes an increase in endogenous amino acid release from the striatum and ventromedial nucleus of the hypothalamus *in vitro*

349P **Pinnock RD*, Woodruff GN & Boden PR**
Cholecystokinin excites dorsal raphé neurones via a CCK_A receptor

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Cholecystokinin modulates the release of dopamine from the anterior and posterior nucleus accumbens by two different mechanisms

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Electroconvulsive shock increases dopamine D_1 receptor-mediated adenylate cyclase activity in rat striatal homogenates

352P **den Daas I*, Tepper PG & Horn AS**
The activity of the dopamine agonist N-0437 and eight ester prodrugs after oral and transdermal administration in the 6-OHDA turning model

353P **Rivest R*, Jolicoeur FB & Marsden CA**
Atypical neuroleptic-like properties of neurotensin: an *in vivo* neurochemical study

354P **Hitchcott PK*, Zharkovsky A & File SE**
Acute CGS 8216 reverses, and concurrent verapamil prevents, diazepam withdrawal-induced anxiety in rats

355P **Kenny BA*, MacKinnon AC, Kilpatrick AT & Spedding M**
Species differences in the sensitivity to [^{3}H]-PK11195, a potential marker of neuronal damage

356P **Luscombe GP*, Mazurkiewicz SE & Buckett WR**
Evaluation of tricyclic antidepressants in the elevated plus-maze in rats: anxiolytic effect of dothiepin and doxepin

357P **Wright IK*, Ismail H, Upton N & Marsden CA**
Effect of isolation-rearing on performance in the elevated plus-maze and open field behaviour

358P **Connaughton S* & Docherty JR**
Evidence for subtypes of peripheral prejunctional α_2-adrenoceptors

359P **Mallard NJ, Marshall RW* & Spriggs TLB**
Neuronally-released noradrenaline acts at different α_1-adrenoceptors to exogenous noradrenaline in rat vas deferens

360P **Spraggs CF* & Patel M**
Functional comparisons of L-365,260 and other gastrin/CCK receptor antagonists in gastric mucosa and ileum *in vitro*

361P **Bobinski H*, Hadley H, Miller M, Balment RJ & Weston AH**
Effects of erythrocyte extracts from genetically hypertensive rats on vascular smooth muscle and on calcium uptake by human red cells

362P **Offer GJ*, Chilvers ER & Nahorski SR**
Muscarinic receptor-mediated phosphoinositide hydrolysis is inhibited by isoprenaline and IBMX in bovine tracheal smooth muscle

363P **Chilvers ER*, Challiss RAJ, Batty IH & Nahorski SR**
Is the transient accumulation of inositol (1,4,5) trisphosphate in muscarinic-stimulated airway smooth muscle due to transient formation or enhanced metabolism?

364P **Chilvers ER*, Giembycz MA, Challiss RAJ & Nahorski SR**
Zaprinast does not influence methacholine-induced tone or inositol (1,4,5) trisphosphate accumulation in bovine airway smooth muscle

365P **Watson N*, Barnes PJ & Maclagan J**
Evidence for an effect of methoctramine on ganglionic nicotinic cholinoceptors in the guinea-pig trachea *in vitro*

366P **Michel AD* & Whiting RL**
Gallamine as a pharmacological tool to aid the identification of muscarinic receptor subtypes

367P **Murray MA*, Foster RW & Small RC**
Effects of the K^+ channel openers cromakalim and RP 49356 on the cyclic nucleotide content of guinea-pig isolated trachealis muscle

368P **Chopra LC*, Twort CHC & Ward JPT**
Effects of BRL 38227 on calcium uptake by intracellular stores in cultured rabbit airway smooth muscle cells

369P **Ward JS, Spicer BA & Taylor SG***
Effects of lemakalim (BRL 38227) and salbutamol on hyperresponsive lung parenchyma

370P **Taylor SG* & Spicer BA**
Effects of 5-HT and carbachol on small and large pulmonary arteries from hyperresponsive and normal rats.

371P **Gunn LK* & Piper PJ**
Limitations of the co-axial bioassay

372P **Herbert CA*, Edwards D, Boot JR& Robinson C**
Eosinophil activation elicits an enhancement in the permeability of the bronchial mucosa to albumin

373P **Herbert CA*, Edwards D, Boot JR & Robinson C**
Modulation of the eosinophil-induced enhancement of bronchial epithelial permeability

374P **Kroegel C*, Chilvers ER, Giembycz MA, Challiss RAJ & Barnes PJ**
Receptor-mediated accumulation of D-myo-inositol (1,4,5,) trisphosphate mass in guinea-pig eosinophils

375P **Giembycz MA*, Kroegel C, Dent G & Barnes PJ**
Induction of thromboxane and E-series prostaglandin biosynthesis in guinea-pig eosinophils stimulated with platelet activating factor

376P **Rabe K*, Giembycz MA, Dent G & Barnes PJ**
Leukotriene B_4 stimulates hydrogen peroxide generation in guinea-pig eosinophils: possible role of protein kinase C

POSTER COMMUNICATIONS

377P **Pangalos MN, Pearson RCA, Francis PT, Middlemiss DN & Bowen DM**
Selective destruction of a sub-population of cortical neurones by suicide transport of volkensin, a lectin from *Adenia volkensii*

378P **Collins GGS & Valentine DJ**
Exogenous glycine and D-serine potentiate an N-methyl-D-aspartate receptor-mediated synaptic potential in mouse olfactory cortex slices

379P **Anson J, Collins GGS & Green SC**
Possible interactions of 1,2,3,4-tetrahydroamino acridine with N-methyl-D-aspartate receptors

380P **Moodley I, Grouhel A, Witko V & Junien JL**
Effects of PCP and sigma ligands on NMDA- and DTG-induced convulsions and mortality in mice.

381P **Black MD, Brotchie JM, Mitchell IJ & Crossman AR**
Potassium thiocyanate-induced increases in EAA receptor binding in the rat visualised by [^{3}H]-L-glutamate involves NMDA receptors in addition to AMPA receptors

382P **Steele JE, Bowen DM, Francis PT, Green AR & Cross AJ**
Spermidine enhancement of [^{3}H]-MK-801 binding to the NMDA receptor complex in human brain

383P **Collins GGS & Prescott S**
A study of the possible mechanisms by which polymixin B selectively antagonizes responses evoked by N-methyl-D-aspartate

384P **Bagetta G, Nistico G & Bowery NG**
Behavioural excitation and neuronal loss induced by intrahippocampal tetanus toxin in rats are prevented by CGP 37849

385P **Brotchie JM, Mitchell IJ & Crossman AR**
Injections of EAA antagonists in the entopeduncular nucleus of the rat alleviate reserpine-induced akinesia by an action at NMDA receptors

386P **Smith AL & McIlhinney RAJ**
Synthesis and characterization of two novel agonists for the kainate receptor

387P **Croucher MJ & Bradford HF**
Anticonvulsant and antiepileptogenic actions of a strychnine-insensitive glycine receptor antagonist in the kindling model of epilepsy

388P **Smart TG, Moss S & Nayeem N**
'Stable' expression of functional α_1 and β_1 subunits for the vertebrate neuronal $GABA_A$ receptor in Chinese hamster ovary cells

389P **Freeman P & Sturman G**
Histamine H_1 antagonists modulate seizure pattern induced by electroshock in mice

390P **Alexander SPH, Hill SJ & Kendall DA**
A23187 and 2-chloroadenosine increase guinea-pig brain histamine-induced inositol phosphates accumulation by different mechanisms

391P **Young NJ, Wilson VG & Kendall DA**
Effects of a crude bovine brain extract of clonidine-displacing substance on [^{3}H]-myo inositol uptake and phosphoinositide hydrolysis in rat brain slices

392P **Jackson HC, Dickinson SL & Nutt DJ**
Non-α_2-adrenoceptor idazoxan binding sites are not involved in the pro-convulsant action of idazoxan

393P **Jones CA**
The effect of methohexitone on the release of [^{3}H]-noradrenaline from rat brain slices

394P **Elliott P & Wallis DI**
Preliminary studies of the action of 5-HT on single lumbar motoneurones in the spinal cord of the neonate rat

395P **Stanton JA, Watling KJ & Beer MS**
The effect of Ca^{2+} and Mg^{2+} ions on the binding of [^{3}H]-Q ICS 205-930 to the 5-HT_3 recognition site in rat brain whole cortex

396P **Hornsby CD, Barnes JM, Barnes NM, Costall B & Naylor RJ**
Pharmacological comparison of the rat and guinea-pig cortical high affinity 5-HT uptake system

397P **Twist EC, Mitchell SN, Corn TH & Campbell IC**
Effect of a chronic ritanserin regimen on amine and metabolite levels in rat frontal cortex and cerebellum

398P **Boyle EA, Davey PD, Marr HE & Blower PR**
The protective effect of BRL 43694 against cytostatic treatment in 10-13 week old ferrets

399P **Gonzalez-Mora JJ, Mas M, O'Connor J, Trout SJ & Kruk ZL**
Qualitative and quantitative estimation of dopamine and 5-HT in mixtures assessed by fast cyclic voltammetry (FCV) and numeric analysis

400P **Hassoni AA, Marsden CA & Crespi F**
In vitro analysis of striatal electroactive compounds with differential pulse voltammetry and carbon fibre micro electrodes

401P **De Souza RJ, Sarna GS, Green AR, Robinson TN & Cross AJ**
Extracellular dopamine concentration in rat striatum following tetrahydroaminoacridine administration

402P **Barnes NM, Cheng CHK, Costall B, Gerrard PA & Naylor RJ**
Reversible dopamine depletion following unilateral infusion of MPTP in the common marmoset

403P **Browning JG, Redfern RJ, Luscombe GP, Hutchins LJ, Heal DJ & Buckett WR**
Presynaptic dopamine uptake sites labelled by [^{3}H]-GBR 12935: effects of unilateral 6-hydroxytryptamine lesions of rat substantia nigra

404P **Heal DJ, Cheetham SC, Viggers JA, Slater NA, Martin KF, Phillips I, Browning JG, Redfern RJ, Luscombe GP & Buckett WR**
A comparison of the monoamine reuptake inhibition and α_1- and muscarinic receptor binding properties of dothiepin, its metabolites and other antidepressants

405P **Martin KF, Phillips I, Cheetham SC, Heal DJ & Buckett WR**
Only antidepressants with marked effects on central dopamine function decrease rat striatal D_2 receptor number

406P **Naylor RJ & Rudd JA**
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