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1P ALTERED INOSITOL PHOSPHATE METABOLISM IN TAIL ARTERIES IN SPONTANEOUSLY HYPERTENSIVE RATS

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A change in polyphosphoinositide (PPI) metabolism, the signal-transduction mechanism mediating the contractile responses to noradrenaline (NA, Abdel-Latif, 1986), manifest as an enhanced inositol triphosphate (IP_3) accumulation, occurs in spontaneously-hypertensive rats (SHR) to NA (Jenkinson et al, 1990). The site of this alteration has now been investigated.

NA-stimulated PPI metabolism in tail artery segments (1cm, 5mg wet wt.) from age-matched Wistar Kyoto (WKY) and spontaneously-hypertensive rats (SHR), was estimated by measuring the accumulation of inositol monophosphate (IP_1), inositol bisphosphate (IP_2) and IP_3 (Akhtar and Abdel-Latif, 1984). The rates of formation and decay, after adding prazosin (10^{-6}M) to terminate NA-stimulated PPI hydrolysis of these IP's were measured.

IP_3 accumulation increased significantly more in tissues from SHR than in those from WKY at each effective concentration of NA. This increase was parallelled by an equivalent reduction in IP_1, consistent with the lack of any significant difference in total IP accumulation between the two populations. Time course studies suggest that the enhanced NA-evoked IP_3 accumulation, in arteries from SHR rats, is due to a reduced rate of metabolism of both IP_3 and IP_2 rather than to a greater formation of IP_3 from PPI's. This enhanced IP_3 accumulation may result in an increased calcium mobilisation and so account for the increased contractility to NA of such arteries.

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2P INVOLVEMENT OF NITRIC OXIDE IN INHIBITORY JUNCTION POTENTIALS IN CANINE PROXIMAL COLON

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Nitric oxide has been implicated in non-adrenergic, non-cholinergic (NANC) relaxation in a variety of visceral muscles. The underlying mechanism of NANC relaxation is hyperpolarization of smooth muscle membrane (i.e. inhibitory junction potentials; IJPs). Experiments were performed to study the basis of NANC IJPs in circular muscle cells of the canine proximal colon. Cells near the myenteric surface of the circular smooth muscle layer were specifically studied using intracellular microelectrodes. The effects of an inhibitor of NO-synthase, L-N^G-nitroarginine methyl ester (L-NAME, 100μM), on the nerve-mediated IJPs, and the ability of authentic NO and the NO carrier, nitrosocysteine (cys-NO; synthesized according to method of Field et al.; 1978) to mimic NANC hyperpolarizations were evaluated. Circular muscle cells in this region had average resting potentials of -43 ± 2 mV (n=11). NANC nerve stimulation (0.1 Hz) produced IJPs averaging 24.4 ± 4 mV in magnitude and 2.6 ± 0.2 s (n=7) in duration. The NO-synthase inhibitor, L-N^G-nitroarginine methyl ester (L-NAME, 100μM), reduced the IJP size to 3 ± 1 mV ($P<0.05$, n = 7). This inhibition was reversed by L-arginine (1 mM, n = 8), but not by D-arginine (1 mM, n=3). NO and L-cys-NO (0.1-100μM) caused dose-dependent hyperpolarizations with similar potency. Lower concentrations of these drugs mimicked IJPs elicited by low frequency stimulation (0.1 Hz). Higher concentrations of NO and L-cys-NO mimicked responses elicited by NANC nerve stimulation at higher frequencies (1 Hz). *oxy*-Haemoglobin (2%), a scavenger of NO, blocked the effects of NANC nerve stimulation, NO and L-cys-NO. These data support the hypothesis that NANC neurotransmission is mediated by NO, or an NO related compound such as a nitrosothiol. In vascular smooth muscle the effects of NO are mediated by increased production of cGMP, and NANC nerve stimulation has also been shown to increase cGMP in some smooth muscles. Therefore, the membrane permeable cGMP analogue, 8-Br-cGMP, was employed to determine whether cGMP may be involved in the production of hyperpolarization. 8-Br-cGMP mimicked the hyperpolarizations caused by sustained NANC stimulation and higher concentrations of NO and L-cys-NO. IJPs are known to be due to an increase in potassium conductance in several smooth muscles. The effects of NO were tested on Ca^{2+}-activated K channels using on-cell patch clamp recordings of single channels. At 36°C, and a holding potential of +50 mV, NO (50μM) caused an increase in the open probability of Ca^{2+}-activated K channels from 0.27 ± 0.24 to 3.2 ± 1.2 (n = 4). These results suggest that NO, perhaps liberated from a conjugate such as L-cys-NO, mediates NANC IJPs colonic circular muscles via a cGMP-dependent mechanism. NO increased open probability of Ca^{2+}-activated K channels which may provide a mechanism for IJPs.

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3P NON-ADRENERGIC, NON-CHOLINERGIC INHIBITION AND REBOUND EXCITATION IN CANINE PROXIMAL COLON ARE MEDIATED BY NITRIC OXIDE

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Electrical slow waves originate from pacemaker cells along the submucosal surface of the circular muscle layer in the canine proximal colon. These events determine the timing and force of phasic contractions. One of the important regulators of electrical slow waves in the colon is non-adrenergic, non-cholinergic (NANC) nerves. The present study investigated the role of nitric oxide (NO) in NANC nerve-mediated responses at the submucosal border.

Circular smooth muscle cells at the submucosal border had resting membrane potentials of -81 ± 2 mV and exhibited spontaneous rhythmic depolarizations (slow waves). Brief stimulation (3 pulses at 20 Hz) of NANC nerves, delivered at the plateau of the slow wave caused premature repolarization. Longer trains of pulses (50-200 pulses at 5-20 Hz) also inhibited slow waves during the period of stimulation, but subsequent to the inhibited slow waves a slow wave of significantly enhanced duration was observed. This post-stimulus excitation has been termed "rebound excitation", and it occurs in many gastrointestinal muscles. Exogenous NO mimicked NANC nerve stimulation. Lower concentrations of NO (0.1-1 μM) produced a decrease in slow wave amplitude and duration, and higher concentrations (3-30 μM) caused an initial inhibitory response followed by rebound excitation. The effects of NANC nerve stimulation were blocked by the NO-synthase inhibitor, L-N^G-nitroarginine methyl ester (L-NAME, 100μM) and by TTX (1μM). Responses to exogenous NO were unaffected by either L-NAME or TTX. *oxy*-Haemoglobin (1%), but not *met*-haemoglobin (1%), blocked responses to NANC nerve stimulation and NO. The cyclooxygenase inhibitor, indomethacin (10 μM), had no effect on the initial inhibition of slow waves caused by NANC nerve stimulation, but reduced or abolished the rebound excitation.

These results suggest the involvement of NO in NANC nerve-mediated regulation of slow waves in the canine proximal colon. The initial inhibition of slow waves may be directly mediated by NO, but the rebound excitation may be due to NO-mediated release of a prostanoid from a non-neuronal source.

4P INFLUENCE OF N^G-NITRO-L-ARGININE METHYL ESTER ON VAGALLY-INDUCED GASTRIC RELAXATION IN THE RAT

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In vitro studies suggest that nitric oxide (NO) and vasoactive intestinal polypeptide (VIP) are co-transmitters of the inhibitory non-adrenergic non-cholinergic (NANC) neurones of the rat gastric fundus (De Beurme & Lefebvre, 1988; Li & Rand, 1990; Boeckxstaens et al., 1991). NO might be responsible for the initial rapid relaxation induced by transmural stimulation of the NANC neurones; this rapid response is indeed resistant to VIP antiserum. The vagal nerve contains preganglionic fibers synapsing with the gastric inhibitory NANC neurones. We therefore investigated the influence of the NO biosynthesis inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) on short-lasting vagally induced gastric relaxation in the rat. Male Wistar rats were anaesthetized with pentobarbital (60 mg kg^{-1} i.p.). Intragastric pressure was measured via an intragastric balloon introduced via the esophagus. Blood pressure was measured from the right carotid artery and i.v. injections were given via the right external jugular vein. The cervical vagal nerves were bilaterally sectioned and the peripheral cut end of the left vagus was placed on a bipolar electrode. Peripheral vagal stimulation (10 Hz, 10 V, 1 ms for 20 s) induced a biphasic response : a short-lasting increase (0.5 ± 0.1 cm H_2O) followed by a more pronounced decrease (2.3 ± 0.3 cm H_2O, n = 6) in intragastric pressure; the response was reproducible. L-NAME (1-30 mg kg^{-1} i.v.) increased arterial blood pressure but had no influence on basal intragastric pressure. L-NAME, 1 mg kg^{-1} i.v., had no influence on the vagally induced gastric response while 10 and 30 mg kg^{-1} i.v. L-NAME significantly changed it : the initial increase in intragastric pressure was enhanced while the decrease in intragastric pressure was reduced or abolished (for L-NAME 30 mg kg^{-1} i.v. : increase from 1.2 ± 0.3 to 3.9 ± 1.1 cm H_2O, decrease from 2.6 ± 0.7 to 0.7 ± 0.5 cm H_2O, n = 6, $p < 0.05$, signed-ranks test). Phenylephrine, in a dose (10 μg kg^{-1} min^{-1}) inducing the same pressor response as 30 mg kg^{-1} i.v. L-NAME, did not influence the vagal gastric response. A primed infusion of L-arginine (300 mg kg^{-1} bolus, 100 mg kg^{-1} h^{-1} for 30 min) prevented the effect of 10 mg kg^{-1} i.v. L-NAME but not that of 30 mg kg^{-1}. After injection of both atropine (1 mg kg^{-1} i.v.) and L-NAME (30 mg kg^{-1} i.v.), the vagally induced increase in intragastric pressure was abolished while the vagally induced decrease in intragastric pressure was maintained.
It is concluded that vagal stimulation at the parameters used activates both excitatory cholinergic and inhibitory NANC neurones in the stomach. The results are consistent with NO being involved in gastric relaxation during vagal stimulation but also indicate that, besides NO, another inhibitory NANC neurotransmitter is released.

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5P NITRIC OXIDE IN INHIBITORY TRANSMISSION IN THE GUINEA-PIG LOWER OESOPHAGEAL SPHINCTER

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In the guinea pig lower oesophageal sphincter (gpLOS) stimulation of non-adrenergic non-cholinergic (NANC) nerves produces relaxation which is tetrodotoxin sensitive. Vasoactive intestinal peptide (VIP) has been proposed as the transmitter (Goyal & Rattan, 1980), but since nitric oxide (NO) has been associated with NANC inhibitory responses in the canine ileocolic junction (Bult et al, 1990), it was decided to examine the effects of NO on the gpLOS.

The circular muscle of the gpLOS was dissected out and a strip (1 x 0.5cm) set up for isometric tension recording in a water-jacketed (10ml) organ bath at 36±0.5°C; atropine and phentolamine (both at 10^{-6}M) were present throughout. Strips were stimulated using Ag/AgCl ring electrodes (0.5cm o.d.) with square wave pulses at supramaximal voltage (20-30V 0.05ms, 4 at 1Hz). Following the spontaneous development of tone, inhibitory NANC nerve stimulation produced a stimulus- and frequency-dependent relaxation with a rebound contraction. The optimum stimulation frequency was 1-2Hz and the relaxation was sensitive to TTX ($5x10^{-7}$M-10^{-6}M). NO (1-$6x10^{-6}$M) and ATP (1-$6x10^{-5}$M) each produced concentration dependent relaxation in the gpLOS. N^{G}-nitro-L-arginine methyl ester (L-NAME 10^{-4}M), an inhibitor of NO synthesis, reduced but did not completely abolish the inhibitory response to NANc nerve stimulation. The NO precursor L-arginine ($2x10^{-4}$M) produced some recovery from the effects of L-NAME. D-NAME (10^{-4}M) was without effect on the response to nerve stimulation. Oxyhaemoglobin ($1x10^{-5}$M) reduced responses to nerve stimulation and to NO ($6x10^{-6}$M) but not to those of ATP. The bee venom, apamin ($4x10^{-7}$M) abolished the responses to ATP (1-$6x10^{-5}$M) but not to NO. Apamin partially reduced the response to NANC stimulation, and inhibitory responses to NANC stimulation which had been reduced with L-NAME ($1x10^{-4}$M) or with oxyhaemoglobin ($1x10^{-5}$M) were abolished by the further addition of apamin ($4x10^{-7}$M).

The results demonstrate that NO may be released by the NANC nerves to produce relaxation, but it is not the sole inhibitory agent released.

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6P NITRIC OXIDE IN INHIBITORY TRANSMISSION IN THE GUINEA-PIG INTERNAL ANAL SPHINCTER

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Stimulation of inhibitory non-adrenergic, non-cholinergic (NANC) nerves produces a tetrodotoxin (TTX)-sensitive membrane hyperpolarisation and relaxation of the guinea pig internal anal sphincter (gpIAS) which is mediated by an increase in gK^{+} (Lim & Muir, 1985). The nature of the transmitter(s) is unknown though the relaxant effects of ATP resemble those produced by NANC nerve stimulation (Lim & Muir, 1985).

The recent involvement of nitric oxide (NO) in NANC nerve-mediated inhibitory responses in sphincteric smooth muscle (Bult et al., 1990) prompted the present comparison of the effects of stimulation of these nerves with those of NO and ATP in the gpIAS. The circular muscle of the gpIAS was dissected (Lim & Muir, 1985) and strips (2 x 0.5cm) set up for isometric tension recording in a jacketed (10ml) organ bath at 36±0.5°C; atropine and phentolamine (each 10^{-6}M) were present throughout. Strips were stimulated using Ag/AgCl ring electrodes (0.5cm o.d.) by square wave pulses at supramaximal voltage (20-30V 0.05ms, 4 at 1-32Hz). Following the spontaneous development of tone, inhibitory NANC nerve stimulation produced a stimulus- and frequency-dependent (optimum 1-2Hz), TTX ($5x10^{-7}$M-10^{-6}M)-sensitive relaxation and a rebound contraction. ATP (1-$6x10^{-5}$M) and NO (as a solution of the gas, equivalent to 1-$6x10^{-6}$M, prepared as described by Gillespie & Sheng, 1988) each relaxed the gpIAS in a concentration-dependent manner. The inhibitory NANC response (4 pulses 2-32Hz 0.05ms supramaximal 20-30 volts) was significantly ($p<0.01$) reduced (by some 54-66%) but not abolished, by NΩ-Nitro-l-Arginine methyl ester (L-NAME 10^{-4}M) which inhibits NO synthesis. This inhibition of the neuronal response was partially, but not completely, restored by L-arginine ($2x10^{-4}$M), a precursor of NO. D-NAME (10^{-4}M) and the bee venom, apamin ($4x10^{-7}$M), were each ineffective antagonists of the neuronal response. The response to ATP (1-$6x10^{-5}$M) (but not that to NO, $6x10^{-6}$M which was unaffected) was abolished by apamin ($4x10^{-7}$M).

Responses to inhibitory NANC nerve stimulation (4 pulses, 1-4Hz, 0.05msec supramaximal voltage) were inhibited by oxyhaemoglobin ($5x10^{-6}$M), further reduced by the additional presence of L-NAME ($1x10^{-4}$M) and abolished by the further addition of apamin ($4x10^{-7}$M). Responses to NO were abolished but those to ATP ($6x10^{-5}$M) were not significantly affected by oxyhaemoglobin ($1x10^{-5}$M). The results demonstrate that inhibitory nerve stimulation releases at least two relaxant substances in the gpIAS, one is NO, the other, as yet, unknown.

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7P CHARACTERISATION OF NO-SYNTHASE FROM NANC NERVE-CONTAINING TISSUES: RAT ANOCOCCYGEUS AND BOVINE RETRACTOR PENIS MUSCLES

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The inhibitory responses to field stimulation in the rat anococcygeus (RAC) and bovine retractor penis (BRP) muscles are mediated by the release of non-adrenergic non-cholinergic (NANC) neurotransmitter(s) which is most probably nitric oxide (NO) or a NO-releasing substance (Gillespie & Sheng,1990; Martin et al., 1991). Here we report that NO-synthase activity is present in both soluble and particulate fractions isolated from these tissues.

RAC or BRP muscles were removed from the animals and frozen in liquid nitrogen. Muscles were homogenized in ice-cold Tris/HCl buffer as previously described (Schmidt et al.,1991). Samples were centrifuged (100,000 x g, 60 min) and both the supernatant and pellet fractions collected. The pellet was washed with 1 M KCl and solubilized with the detergent CHAPS (Förstermann et al.,1991). For further purification, the cytosolic or CHAPS-solubilized particulate fractions from BRP were loaded onto a DEAE Sephacel column and eluted with 0.3 M NaCl. The NO-synthase activity in each fraction was assayed by the accumulation of cGMP in rat fetal lung fibroblasts (RFL-6 cells, Ishii et al., 1989), or by the formation of L-citrulline from L-arginine (Schmidt et al., 1991). L-arginine (10 or 100 μM), NADPH (0.1 or 1 mM), 5,6,7,8-tetrahydrobiopterin (BH_4, 3 μM), and calmodulin (30 U/ml) were added to all assays except where indicated.

The activities of crude soluble and particulate fractions from RAC, as assayed by citrulline formation, were 12.4±1.5 pmol/mg/min (n=9) and 33.1±10 (n=6), respectively. Soluble and particulate fractions from BRP, which were partially purified over the DEAE column, had activities of 3.7±0.3 pmol/mg/3 min (n=3) and 30.9±2.0 (n=3), as assayed on RFL-6 cells. The characterization of NO-synthase from BRP is shown in Table 1 (similar results were obtained from fractions of RAC).

Table 1.

Fraction	Ca^{2+}-free/EGTA (1 mM)	NADPH-free	BH_4-free
	(% of enzyme activity from DEAE column)		
soluble	0 (3)	36.6±6.1 (3)	19.9±3.9 (3)
particulate	1.2±1.2 (3)	21.2±2.8 (3)	23.2±3.1 (3)

The enzymes from all fractions are NADPH, BH_4 and calcium activated. The activity of all enzyme fractions were inhibited by L-arginine analogues, N^G-methyl-L-arginine and N^G-nitro-L-arginine (n=3). Fractions prepared from rat vas deferens, which does not display a NO-related NANC response, were inactive. Thus, soluble and particulate isoforms of NO-synthase are located in NO-related NANC containing tissues and are presumably responsible for the synthesis of NANC neurotransmitter.

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8P ROLE OF ENDOGENOUS VASOACTIVE INTESTINAL POLYPEPTIDE AND NITRIC OXIDE IN PRE- AND POST-GANGLIONIC CHOLINERGIC NEUROTRANSMISSION IN GUINEA-PIG TRACHEA

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Vasoactive intestinal polypeptide (VIP) and nitric oxide (NO) have been proposed as the neurotransmitters involved in the NANC-inhibitory (NANC-i) system in guinea-pig trachea (Tucker et al., 1990, Li and Rand 1991), however the release of NO from these nerves has not been directly demonstrated. The present study using the isolated innervated tracheal tube preparation of the guinea-pig investigates whether (i) VIP and/or NO can be released during pre-ganglionic stimulation of vagal nerve fibres and (ii) whether the modulation, by NO, of contractions of the trachea induced by stimulation of post-ganglionic intramural nerves is dependant on an intact epithelium. The effect of the peptidase α-chymotrypsin which degrades VIP and the NO synthesis inhibitor L-N^G-nitro arginine (L-NOARG) were investigated, on contractions of the guinea-pig trachea induced by pre-ganglionic vagal nerve stimulation (PGS- 30V, 30Hz, 0.2ms), post-ganglionic intramural nerve stimulation (TMS- 60V, 30Hz, 0.2ms) and exogenously applied acetylcholine (ACh). The effect of epithelial removal on the response to L-NOARG during TMS was determined.

Tracheas with vagi and recurrent laryngeal nerves intact were removed from male Dunkin Hartley guinea-pigs (250-350g) killed by cervical dislocation. In some tracheas the epithelial surface was removed by gentle running (confirmed histologically). Tracheas were mounted on a stimulating electrode and placed in an organ bath containing Kreb's Henseleit buffer oxygenated with 95% O_2: 5% CO_2, maintaned at 37°C. 5μM indomethacin and 1μM propranolol were present in the Kreb's buffer solution to eliminate prostaglandin-induced tone and complications of stimulating sympathetic nerves during TMS. 75μM hexamethonium was also present during TMS to ensure stimulation of post-ganglionic fibres. Neither α-chymotrypsin (2units ml^{-1}) nor L-NOARG (5μM) had any effect on PGS-induced contractions (30V, 0.2ms) in the frequency range 2-50Hz (1 ± 9 and 3 ± 2% facilitation respectively at 8Hz). Both these agents facilitated TMS-induced contractions at all these frequencies (35 ± 9 and 29 ± 8% facilitation respecitvely at 8Hz). The facilitation by L-NOARG was concentration dependant (1-10μM) and was partially reversed by L-arginine but not D-arginine. Contractions induced by exogenous ACh were unaffected by either of these agents. Removal of the epithelium increased the facilitatory effect of L-NOARG on TMS-induced contractions at 4Hz (14 ± 4 intact vs 56 ± 18% epithelial denuded) but had no significant effect at any of the other frequencies (46 ± 12% at 8Hz).

These results suggest that neither VIP nor NO are co-released with ACh onto airway smooth muscle, from cholinergic nerves receiving a pre-ganglionic input via the vagus. Stimulation of intramural nerves however, leads to the release and/or subsequent production of both NO and VIP. The fact that the facilitation of TMS-induced contractions by L-NOARG occurred in preparations devoid of epithelium, suggests that NO is not being released from the epithelial surface as a result of electrical stimulation. These findings do not exclude the possibility that NO is being released or produced by other cell types present in the preparation.

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9P THE ROLE OF ENDOTHELIAL-DERIVED NITRIC OXIDE IN MODULATING THE RENAL VASOCONSTRICTOR RESPONSE TO 5-HT_1-LIKE RECEPTOR AGONISTS

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Renal vasoconstrictor responses in the dog produced by 5-carboxamidotryptamine (5-CT) and sumatriptan (SUM) are mediated by the 5-HT_1-like receptor, and characterised by their transient nature (Cambridge et al., 1991). Recently, Schoeffter & Hoyer (1990) have shown that 5-CT and SUM can stimulate endothelial - dependent relaxation in pig coronary arteries in vitro. We have therefore used N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of enzymic synthesis of nitric oxide from L-arginine, (Rees et al., 1990) to determine if endothelial derived nitric oxide (NO) modulates the renal vasoconstrictor responses to these agonists.

The studies were carried out in pentobarbitone (30 mg kg^{-1}) anaesthetised dogs, instrumented to measure mean arterial blood pressure (MBP) and renal vascular conductance (RC), and pretreated with ketanserin (0.1 mg kg^{-1}) and MDL-72222 (0.5 mg kg^{-1}), to block 5-HT_2 and 5-HT_3 receptors respectively. L-NAME (10 mg kg^{-1} iv) produced a significant increase in MBP, (from 110±3 to 141±5 mmHg) and significantly reduced the hypotensive response to acetylcholine (ACh, 0.3 to 3.0 µg kg^{-1}), as shown in Table 1. L-NAME also produced a significant decrease in RC (from 1.43±0.19 to 0.83±0.09 ml min^{-1} $mmHg^{-1}$) but had no effect on the recovery response to a short period of mechanical renal artery occlusion (15 seconds) or the decrease in RC produced by angiotensin II (AII, 0.1 µg kg^{-1} iv). However L-NAME significantly augmented the decrease in RC produced by intra-left atrial administration of 5-CT, (0.03 to 0.3 µg kg^{-1}) and SUM, (1 to 10 µg kg^{-1}), as shown in Table 1, and these effects of L-NAME could be completely reversed by the administration of L-arginine (1000 mg kg^{-1} iv).

Table 1. The effects of L-NAME on (a) the hypotensive response to ACh and the RC response to (b) 5-CT and (c) SUM. Data are shown as mean ± s.e. of mean (n = 4 to 6) of the area under curve (AUC) for each response measured by computerised planimetry (arbitrary units). *Signifies significant difference from control ($P < 0.05$) by the non-parametric Wilcoxan signed rank one-tailed test.

(a) Hypotensive response (AUC) to ACh			(b) RC response (AUC) to 5-CT			(c) RC response (AUC) to SUM		
Dose (µg kg^{-1})	Control	L-NAME	Dose (µg kg^{-1})	Control	L-NAME	Dose (µg kg^{-1})	Control	L-NAME
0.3	235±22	115±21*	0.03	385±70	945±398	1.0	180±64	331±75*
1.0	297±25	166±9*	0.1	455±43	2650±808 *	3.0	288±101	1179±427*
3.0	394±39	206±17*	0.3	651±101	2390±637*	10.0	715±415	1605±757*

The enhanced renal vasoconstrictor effects of these 5-HT_1-like receptor agonists produced by L-NAME suggests that NO release limits this response. However the observation that L-NAME has no effect on the recovery from abrupt mechanical reduction of renal blood flow or on the renal vasoconstriction produced by AII suggests that this NO release is not simply a consequence of a reduction in blood flow. These results therefore indicate that activation of the 5-HT_1-like receptor, which mediates vasoconstriction, also induces endothelial-derived NO release which can modulate the vasoconstrictor responses to 5-HT_1-like receptor agonists in vivo.

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10P SUSTAINED HYPERTENSION IN BRATTLEBORO RATS DURING CHRONIC ORAL INGESTION OF N^G-MONOMETHYL-L-ARGININE

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Recently we reported that acute (9 h) oral ingestion of N^G-monomethyl-L-arginine (L-NMMA) or N^G-nitro-L-arginine methyl ester (L-NAME) caused hypertension in vasopressin-deficient, polydipsic, Brattleboro rats (Gardiner *et al.*, 1990). In the present work we monitored regional haemodynamic changes in conscious Brattleboro rats ingesting L-NMMA over a period of 7 days. Animals were anaesthetized (sodium methohexitone, 60 mg kg^{-1} i.p. supplemented as required) and had pulsed Doppler probes implanted to monitor renal, mesenteric and hindquarters blood flow (Gardiner *et al.*, 1990). At least 7 days later, animals were briefly re-anaesthetized (sodium methohexitone, 40 mg kg^{-1} i.p.) for implantation of catheters. The following day recordings were made of mean arterial blood pressure (MAP), instantaneous heart rate, and renal, mesenteric and hindquarters mean Doppler shift signals (Gardiner *et al.*, 1990). On the first day, animals were given water to drink; at 0700 h on the second day the drinking water had L-NMMA dissolved in it (1 mg ml^{-1}) and this solution was provided for a period of 7 days. At 0700 h on the eighth day plain drinking water was returned to the animals and measurements were continued for a further 2 days.

Nine h after providing L-NMMA solution to drink there was a significant ($P<0.05$, Friedman's test) increase in MAP (25 ± 3 mmHg) and a bradycardia (-25 ± 9 beats min^{-1}). At this time there was an increase in renal flow (14 ± 4%), no change in mesenteric flow (-6 ± 6%), and a reduction in hindquarters flow (-35 ± 5%). Renal conductance was unchanged (-8 ± 2%), but mesenteric and hindquarters vascular conductances were reduced (-23 ± 7 and -47 ± 5%, respectively). Seven days later, MAP was still elevated (35 ± 9 mmHg), but there was no bradycardia (6 ± 19 beats min^{-1}). Renal flow was increased (36 ± 16%), mesenteric flow unchanged (12 ± 10%) and hindquarters flow slightly reduced (-14 ± 5%), relative to baseline; only hindquarters vascular conductance was reduced (-33 ± 7%). Within 9 h after removal of L-NMMA from the drinking water, MAP was not different from the original baseline level. Thus, although hypertension was maintained during chronic ingestion of L-NMMA over 7 days, this effect was not sustained after withdrawal of L-NMMA.

We have preliminary data indicating that ingestion of L-NAME (0.05 mg ml^{-1}) over a period of 4 months causes maintained hypertension, but within 2 days of withdrawing L-NAME, MAP returns to normal levels.

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11P N^G-NITRO-L-ARGNININE METHYL ESTER ENHANCES THE PRESSOR EFFECTS OF ADRENALINE IN CONSCIOUS RATS

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Recently, we found that the hindquarters vasodilator effects of salbutamol were attenuated by L-NAME in conscious rats (Gardiner *et al.*, 1991) consistent with an involvement of nitric oxide in β_2-adrenoceptor-mediated hindquarters vasodilatation. Now we have assessed the haemodynamic effect of (±)-adrenaline infusion (0.5 μg kg^{-1} min^{-1} for 3 min) before, and 60 min after the onset of L-NAME infusion (3 mg kg^{-1} h^{-1} i.v.) in conscious, male, Long Evans rats (n=8). Under sodium methohexitone anaesthesia (60 mg kg^{-1} i.p.) pulsed Doppler probes were implanted to monitor renal (R), mesenteric (M) and hindquarters (H) blood flows. After at least 7 days, under brief anaesthesia (sodium methohexitone 40 mg kg^{-1} i.p.) intravascular catheters were implanted, but experiments did not begin until at least 1 day later. Continuous recordings were made of heart rate (HR), mean arterial blood pressure (MAP) and R, M and H blood flow signals. Vascular conductances were calculated from the latter divided by MAP. The results are summarized in Table 1.

Table 1: Cardiovascular responses to adrenaline infusion in the absence and presence of L-NAME. Values are mean ± s. e. mean.

	HR (beats min^{-1})	MAP (mmHg)	Flow (kHz) R	Flow (kHz) M	Flow (kHz) H	Conductance (kHz $mmHg^{-1}$)10^3 R	Conductance (kHz $mmHg^{-1}$)10^3 M	Conductance (kHz $mmHg^{-1}$)10^3 H
Control	342 ± 11	110 ± 3	11.2 ± 1.9	7.0 ± 0.6	4.5 ± 0.4	102 ± 17	64 ± 6	41 ± 4
+ Adrenaline	423 ± 12*	95 ± 4*	11.2 ± 1.8	5.3 ± 0.4*	10.4 ± 1.2*	116 ± 17*	57 ± 5	110 ± 11*
L-NAME	292 ± 9†	138 ± 7†	11.0 ± 2.2	5.0 ± 0.5†	3.6 ± 0.4	80 ± 16†	38 ± 5†	27 ± 4†
+ Adrenaline	261 ± 17°	146 ± 7*°	10.8 ± 2.0	4.1 ± 0.5*°	5.4 ± 0.8*°	74 ± 14°	29 ± 4*°	39 ± 7*°

* P<0.05 versus baseline (Friedman's test); † P<0.05 versus control (Wilcoxon's test); ° P<0.05 versus adrenaline alone for areas under or over curves (Wilcoxon's test)

Thus, in the presence of L-NAME the depressor response to adrenaline was converted into a pressor response, together with loss of the tachycardia and abolition of the renal and marked attenuation of the hindquarters vasodilatation and an unmasking of a mesenteric vasoconstriction. These results are consistent with an involvement of nitric oxide in the renal, mesenteric and, particularly, hindquarters vasodilator responses to adrenaline under normal conditions. They raise the important possibility that impairment of endothelial function could enhance the hypertensinogenic properties of endogenous adrenaline (Majewski & Rand, 1986).

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12P EDRF RELEASE ACCOUNTS FOR THE DEPRESSANT EFFECT OF THE ENDOTHELIUM ON CONTRACTIONS OF RABBIT ISOLATED BASILAR ARTERY TO 5-HT

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The mechanisms by which the endothelium depresses the contractility of certain blood vessels to exogenous vasoconstrictors are not yet fully understood (e.g. see Connor & Feniuk, 1989). In this study we examined the influence of the endothelium on 5-HT-induced contractions of rabbit isolated basilar artery.

Basilar arteries were removed from pentobarbitone-overdosed (300mg/kg), male New Zealand White or half-lop rabbits (2-4kg), and mounted under a resting tension of 300mg, for the recording of isometric tension in Krebs solution, at 37°C, gassed with 95% O_2/ 5% CO_2. In some experiments endothelial cells were removed mechanically using a fine steel needle. The loss of relaxant response to acetylcholine (10μM), in $PGF_{2\alpha}$-precontracted (3μM) rings, indicated complete removal of the endothelium. 5-HT (1nM-10μM) evoked concentration-related contractions of endothelium-intact (+E) arterial rings. Removal of the endothelium (-E) markedly augmented (Emax) and potentiated (EC50) contractions to 5-HT (see Table 1). N^G-Nitro-L-arginine (L-NOARG, 1 or 30μM), an inhibitor of endothelium-derived relaxing factor (EDRF) synthesis (Moore et al., 1990), increased resting tone, and augmented and potentiated responses to 5-HT in +E, but not -E, rings. These effects were completely inhibited by L-arginine (L-Arg; 1mM) but not by D-arginine (D-Arg; 1mM). Neither glibenclamide (3μM), a putative blocker of the effects of endothelium-derived hyperpolarising factor in rabbit cerebral arteries (Brayden, 1990), nor indomethacin (3μM) altered resting tone or the contractile response to 5-HT in +E rings.

Table 1: Summary of drug effects on 5-HT-induced contractions of rabbit isolated basilar artery

Group	EC50 (nM)		Emax		Group	EC50 (nM)		Emax	
+E	126	(74-214)	34.7	±5.4	+E +L-NOARG (1μM)	43.9	(29.5-65.0)	112.2	±6.6
-E	21.9*	(16.2-29.5)	108.4*	±7.1	+E +L-NOARG (1μM) +L-Arg	203**	(118-348)	32.0**	±7.3
+E+L-NOARG (30μM)	26.3*	(15.8-43.6)	111.8*	±5.5	+E +L-NOARG (1μM) +D-Arg	75.8	(32-177)	125.0	±16.0
-E+L-NOARG (30μM)	14.8*	(10.7-20.4)	102.7*	±8.9					

Values shown are arithmetic means ± sem (expressed as % of response to 40mM KCl) and geometric means (95% confidence intervals), n=4-12. * and ** denote values significantly different from +E and +E +L-NOARG (1μM), respectively (P<0.05; Analysis of variance using Duncan's test for multiple comparisons).

These data strongly suggest that EDRF release fully accounts for the depression of 5-HT-induced contractions in rabbit isolated basilar arteries. It seems likely that a basal release of EDRF underlies this phenomenon, since L-NOARG caused endothelium-dependent contraction. We cannot, however, at this stage eliminate the possibility that 5-HT releases EDRF **per se**.

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13P THE EFFECT OF L-N^G-NITRO ARGININE METHYL ESTER (L-NAME) AND ENDOTHELINS (ET-1 AND ET-3) ON CUTANEOUS BLOOD FLOW IN THE DIABETIC RAT

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The nitric oxide synthesis inhibitor L-NAME (Moore *et al.*, 1990) causes local inhibition of blood flow when injected intradermally (id) and enhances the decrease in blood flow induced by id vasoconstrictors such as ET-1 in the rat (Lawrence & Brain 1991). This implies a role for nitric oxide in the maintenance of cutaneous blood flow. A reduced response to L-NAME in diabetic rats has been reported (Kiff *et al.*, 1991), so we have now studied the effects of L-NAME in the skin of diabetic rats.

Male Wistar rats (150-200g) were made diabetic with intraperitoneal streptozotocin (STZ, 70mg kg^{-1}). Plasma glucose levels, 4 weeks later were: STZ-treated 29.6±1.0 mmol l^{-1}, mean ± s. e. mean n=23; saline-treated 6.6±0.2 mmol l^{-1}, n=20. Basal blood flow at uninjected sites measured by laser Doppler flowmetry (Brain & Williams 1989) and at Tyrode-injected sites (100µl/site) measured by ^{133}Xenon clearance (Lawrence & Brain 1991) was similar in both groups (laser blood cell flux, increases linearly with blood flow, diabetic 80.8±10.4 flux n=6, normal 89.2±5.7 flux n=6; % ^{133}Xenon cleared by 15 min after id injections, diabetic 21.0±1.4% n=23, normal 21.3±1.2% n=20).

The effects of L-NAME, ET-1 and ET-3 were investigated by ^{133}Xenon clearance over 15 min after id injections. L-NAME inhibited skin blood flow in both groups, but the inhibition was significantly smaller in diabetic rats, as shown in Table 1. The enantiomer D-NAME had no effect on blood flow in either group. ET-1 and ET-3 induced a dose-dependent constriction; however the responses to higher doses were significantly reduced in diabetic rats. L-NAME enhanced the constrictor effect of endothelin in both groups but the cutaneous vasoconstriction was also reduced in diabetic rats.

Table 1. Blood flow changes (% decrease compared with Tyrode-injected sites, mean ± s. e. mean)

					+L-NAME (10^{-7} mol/site)		
Agent	mol/site	Normal	Diabetic	n	Normal	Diabetic	n
Tyrode		0	0		55.2±2.6	35.9±8.4*	9
ET-1	$3x10^{-13}$	44.0±8.1	39.4±7.7	9	72.4±6.1	50.6±7.9**	9
ET-1	$3x10^{-12}$	93.2±1.0	73.9±4.2**	9	90.9±1.7	71.1±6.1*	9
ET-3	10^{-12}	7.3±7.7	0.7±5.8	7	55.3±7.1	35.2±5.7*	7
ET-3	10^{-11}	62.4±5.5	40.9±6.7*	7	82.1±3.1	67.3±4.4	7

(* $p<0.05$ and ** $p<0.01$ indicates a significant difference between diabetic and normal rats, modified t-test).

These results suggest that cutaneous blood flow in diabetic rats is similar to that in normal rats. However, the response of the skin microvasculature to L-NAME and the endothelins is different in diabetic rats compared with normal rats.

EL holds a British Heart Foundation Ph.D studentship.

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14P SYMPATHETIC MODULATION OF ACETYLCHOLINE RELEASE FROM RABBIT ATRIA EVOKED BY VAGUS NERVE STIMULATION: A NON-ADRENERGIC FACILITATION AND A NORADRENERGIC INHIBITION

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The rabbit isolated perfused atria preparation with the extrinsic vagus and sympathetic innervation intact has been used to study the mutual interactions between the two branches of the autonomic nervous system by measuring the respective transmitter overflow rates upon electrical nerve stimulation (Habermeier-Muth & Muscholl, 1988). Thus, the stimuli exciting vagus and sympathetic nerves can be applied at selected time intervals which provides an advantage over the common method of field stimulation by which all neurons are excited simultaneously. We have previously shown that sympathetic nerve stimulation (SNS) causes a facilitation of acetylcholine (ACh) overflow evoked by vagus nerve stimulation (VNS) provided that the SNS impulses are applied 19 or 50 ms before the VNS impulses (Muscholl & Habermeier-Muth, 1991). There was no alteration of ACh overflow when the pulse-to-pulse intervals were 7, 132, 350, or 500 ms. The facilitation observed at the impulse distance of 19 ms was unaffected by propranolol, idazoxan, or rauwolscine, suggesting that it was not due to a presynaptic β- or an α_2-adrenoceptor. However, the facilitated ACh overflow was further enhanced in the presence of prazosin revealing an inhibitory α_1-adrenoceptor activated by endogenous noradrenaline (NA).

The present experiments were designed to further characterize the sympathetic modulation of cholinergic transmitter release. Fractional rate of overflow (FRO) of [^{14}C]ACh was determined after labelling the cholinergic nerves with [^{14}C]choline. Overflow of endogenous NA was measured by HPLC and electrochemical detection. 3 VNS periods (2 Hz, 3 min) were carried out at 10 min intervals. SNS (2 Hz, 3 min) applied simultaneously with VNS2, but each impulse placed 19 ms before the VNS impulses, had a facilitatory effect on ACh overflow (FRO, expressed as % of that at VNS1, was 156 ± 11.7 %, n=7; controls in the absence of SNS, FRO=98 ± 5.6 %, n=8, means ± s.e.). Since a prazosin-sensitive adrenoceptor facilitating ACh overflow from the rat heart has been described which is activated by oxymetazoline (Bognar *et al.*, 1990) the latter drug was tested in the present study: 20, 100, or 500 nM had no effect but 2.5 µM enhanced FRO of ACh (155 ± 12.5 %, n=4), an effect that was prevented by prazosin 20 nM (102 ± 14.5 %, n=3), confirming the results of Bognar *et al.* (1990).

Since prazosin enhanced (see above) rather than decreased the FRO of ACh facilitated by SNS, endogenous NA does not appear to mediate the *facilitation*. This is confirmed by the observation that the facilitation of ACh overflow by SNS persisted (FRO = 176 ± 12.6 %, n=5) on atria from reserpine-treated rabbits (3 days each 0.2 mg/kg i.v.; 94 % NA depletion). Nevertheless, the facilitatory factor is released by orthodromic impulses from noradrenergic nerves, as perfusion with guanethidine 2.0 µM abolished both SNS evoked NA overflow and facilitation (FRO=104 ± 4.2 %, n=5), and is supposedly a cotransmitter resistant to the action of reserpine.

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15P THE EFFECTS OF α-METHYL 5-HT ON THE RABBIT ISOLATED RIGHT ATRIUM

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5-hydroxytryptamine (5-HT) is known to stimulate the spontaneously beating rabbit right atrium via an effect on 5-HT_3 receptors (McAdams & Rhodes, 1988). This response to 5-HT is antagonised by the selective 5-HT_3 antagonist, GR38032F (Brittain et al., 1987). The current study demonstrates that the 5-$HT_{1C/2}$ agonist, α-methyl 5-HT (Fozard, 1987) can also produce similar effects to those observed with 5-HT, but that these effects are insensitive to blockade with a variety of 5-HT antagonists.

New Zealand white rabbits (2-3 kg) were killed by pentobarbitone overdose and the right atria removed. The atria were placed on platinum recording electrodes and immersed in a 20ml organ bath containing oxygenated Ringer-Locke solution at 37°C. Atropine (1uM) was present throughout the experiment. Atrial rate was derived electrically using a Lectromed MT6 recorder.

Concentration-response curves to 5-HT, α-methyl-5-HT and 2-methyl 5-HT were generated and the effects of a range of antagonists on the increases in rate were investigated. 2-methyl-5-HT and 5-HT produced graded chronotropic effects (mean±sem EC_{50} 2.9±0.7 & 6.7±0.9 uM respectively). The effects of 5-HT were insensitive to blockade with methiothepin or ketanserin (both 0.1uM; concentration-ratios 1.0±0.2 & 1.0±0.1 respectively). GR38032F (0.1-1.0 nM) produced non-competitive antagonism of responses to 5-HT and 2-methyl-5HT . α-methyl-5-HT also produced graded chronotropic effects (EC_{50} 20.3±5.3 uM), although the onset of this response was much slower than that observed with either 5-HT or 2-methyl-5-HT. Responses to α-methyl-5-HT were insensitive to blockade with either methiothepin or ketanserin. However, unlike 2-methyl-5-HT and 5-HT, responses to α-methyl-5-HT were also insensitive to blockade with GR38032F, indicating a lack of involvement of 5-HT_1, 5-HT_2 or 5-HT_3 receptors. Propranolol (0.1uM) and bethanadine (100uM) produced insurmountable antagonism of the response to α-methyl-5-HT.

These results suggest that α-methyl-5-HT produces a chronotropic effect in the rabbit right atrium that is via a mechanism other than an interaction with 5-HT receptors. One possibility is that the agent is taken-up into the sympathetic nerve terminals and displaces noradrenaline which then produces the increase in rate. The exact mechanism(s) involved are currently under investigation. However, these data do raise a question of the reliability of agents such as α-methyl-5-HT when used as a selective agonist.

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16P DIFFERENTIAL EFFECTS *IN VITRO* OF THE ENANTIOMERS OF Org 20494

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The activities of racemic Org 20494 (4,5-dihydro-6-(6-[2-(piperidin-1-yl)ethyloxy]-benzo[b]thien-2-yl)-5-methyl-3(2H)-pyridazinone HCl), a novel cardiotonic agent, have been described by Cottney et al (1991). It is not unusual that the optical isomers of pharmacologically active agents differ in potency and activity profile. We have examined the activities of the enantiomers of Org 20494 in isolated cardiac and vascular preparations. Methods were as described previously by Walker et al (1991).

The (-) enantiomer displays potent inhibition of rabbit cardiac cyclic nucleotide phosphodiesterase activity (PDE III) with negligible activity as a calcium sensitiser in rat Triton-skinned trabeculae. Conversely, the (+) enantiomer enhances contractile responses in trabeculae both at sub-maximal and maximally activating concentrations of free calcium but is considerably less potent in inhibiting cardiac PDE III. Both compounds exhibit positive inotropic activity in rabbit electrically stimulated papillary muscles and both relax rabbit aortic rings pre-constricted with phenylephrine (0.3 μM) with the (-) enantiomer being more potent in both tissues.

	Triton-Skinned Trabeculae		Cardiac PDE III	Papillary Muscle	Aorta
	submaximal (pCa > 5.0) EC_{25} (μM)	maximal (pCa 4.19) % increase at 500 μM	IC_{50} (μM)	EC_{50} (μM)	EC_{50} (μM)
(-) Org 20494	>1000 (18%)	10±2	0.08±0.05	50±12	1.[illegible]0.4
(+) Org 20494	100±36	24±4	14.8±3.5	>50 (15%)	10±1

These results illustrate that relatively minor stereochemical changes to cardiotonic agents may markedly influence their mechanisms of action.

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17P POSITIVE INOTROPIC, Ca^{2+}-SENSITIZING AND CYCLIC NUCLEOTIDE PHOSPHODIESTERASE (PDE) ISOENZYME INHIBITORY EFFECTS OF Org 30029 IN HUMAN ATRIAL MYOCARDIUM

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Previous studies using animal tissues have identified Org 30029 as a potentially promising cardiotonic agent (Cottney et al., 1990). The aim of the present study was to examine the pharmacological and biochemical effects of Org 30029 in human atrial myocardium. Human atrial appendages were obtained from patients undergoing coronary by-pass surgery. The inotropic activity was examined in isolated trabeculae preparations suspended in Krebs-Henseleit solution (32°C) and stimulated at 1 Hz. Ca^{2+}-sensitizing activity was examined in Triton X-100 treated atrial trabeculae as described by Cottney et al. (1990), at a pCa of 5.5, and the drug concentration (EC_{25}), producing 25% of the maximum response to Ca^{2+} determined. PDE isoenzymes were essentially prepared as described by Shahid and Nicholson (1990).

Org 30029 (9-278 μM) produced concentration-dependent positive inotropic (EC_{50}: 185±53 μM, n=3-4) and Ca^{2+}-sensitizing (EC_{25}: 223±22 μM, n=3) responses in human isolated atrial trabeculae. The positive inotropic response evoked by Org 30029 at 278 μM (4-5 fold increase in contractile force) was similar to the maximum response produced by isoprenaline (EC_{50}: 31±12 nM, n=3-4).

Four peaks of PDE activity were observed in human right atrial extract, after high resolution ion-exchange separation on a Mono Q column. The first peak was stimulated by Ca^{2+}/calmodulin (PDE I); the cyclic AMP activity of peak two, was stimulated by 1 μM cyclic GMP (PDE II); the third peak was relatively insensitive to Ca^{2+}/calmodulin or cyclic GMP but selectively inhibited by rolipram (IC_{50}: 6.9 μM) and, is thus, predominantly PDE IV; the cyclic AMP activity of the fourth peak was inhibited by 1 μM cyclic GMP and Org 9935 (IC_{50}: 0.1 μM) which is a potent and highly selective inhibitor of PDE III (Shahid et al., 1991). Org 30029 selectively inhibited the cyclic AMP (1 μM) activity of human atrial PDE III (IC_{50}: 67 μM) and PDE IV (IC_{50}: 17 μM) whilst exerting only weak effects on PDE I and PDE II (IC_{50} > 250 μM).

In conclusion, Org 30029 increases contractile force in human isolated cardiac muscle and the underlying mechanism of action involves both Ca^{2+}-sensitization of contractile proteins and selective inhibition of PDE III and PDE IV.

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18P COMPARATIVE EFFECTS OF FLOSEQUINAN AND MILRINONE ON THE CONTRACTILE ACTIVITY OF CARDIAC MUSCLE FIBRES FROM PATIENTS WITH AND WITHOUT HEART FAILURE

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Flosequinan (F) is a novel arterial-venous vasodilator, currently undergoing clinical development for treating heart failure. Recent clinical reports have created controversy regarding the potential for F to exert a direct effect on cardiac muscle contractile function (Riegger, et al., 1990, Corin et al., 1991). In the present study the inotropic effects of F and the reference cardiotonic milrinone (M) were evaluated using isolated human right atrial trabeculae obtained from patients undergoing cardiac surgery. The existence of heart failure (HF) or not (non-HF) was assessed by the attending physician or surgeon. Only fibres with a 50% increase in tension development in response to exogenous $CaCl_2$ were included in the study. Interactions between F, M and other drugs were determined as described previously (Weishaar, et al., 1990). Both M and F significantly ($p<0.05$) increased the contractility of fibres from non-HF patients, with EC_{25} values of 1.8 μM and 32 μM, respectively (n=8-9 fibres). Pretreatment with 1 μM of the muscarinic receptor agonist carbachol significantly ($p<0.05$) attenuated the response to both compounds. Pretreatment with EC_{40} concentrations of F or M also significantly potentiated the response to the beta-receptor agonist isoproterenol (n=5-11 fibres for each experimental condition). Interestingly, pretreatment with a threshold concentration of the adenylate cyclase stimulator forskolin (FOR) significantly potentiated the response to M but not F (n=5-7 fibres for each experimental condition). In fibres isolated from 7 of the 20 HF patients, the response to F was significantly reduced or abolished altogether compared to fibres from non-HF patients. In contrast, no differences were observed in the response of cardiac fibres from HF patients to M. The response to M was also not altered in fibres from the F-insensitive HF patients. In fibres from F-sensitive HF patients, F failed to produce a significant inotropic response at a concentration comparable to the peak plasma level in HF patients (10 μM), although the response still occurred at higher concentrations. Thus this study demonstrates that F has weak positive inotropic activity relative to M. However, the lack of synergy between F and FOR and the finding that a subset of HF patients exists in which the inotropic response to F but not M is attenuated, suggests that the two agents are acting via different mechanisms.

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19P EFFECTS OF UK-66914 ON ISCHAEMIA-INDUCED AND REPERFUSION-INDUCED ARRHYTHMIAS IN RAT

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UK-66914 inhibits cardiac delayed rectifying K+ current (IK), prolongs QT interval and ameliorates ventricular arrhythmias in the dog (Gwilt et al., 1988; 1991). To examine the hypothesis that these effects are interdependent, we performed experiments using rat, a species deficient in functional cardiac IK (Tande et al., 1990). The hypothesis predicts that UK-66914 should have no effect on QT or ventricular arrhythmias in the rat.

Left regional ischaemia was induced in rat hearts perfused with solution containing (in mM) NaCl 118.5, $NaHCO_3$ 25.0, KCl 3, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.4 and glucose 11.1, pH 7.4, 37°C or similar solution modified by addition of 0.3 or 1 μM UK-66914. Hearts were randomized to treatment in lines. The incidences (%) of ventricular tachycardia *(VT)* and fibrillation *(VF)* were recorded during 30 min regional ischaemia and 5 min reperfusion. QT interval measured before and during ischaemia *(Isch.)* was corrected according to Bazett's formula ($QTc = QT/\sqrt{RR}$). Data analysis was blinded. Codes were broken after 6 lines (i.e., n=6/group) and the study terminated if there were no statistically significant differences ($p<0.05$) between groups (Chi^2 or ANOVA).

Table 1.	Ischaemia-induced		Reperfusion-induced		QTc (ms)		
	VT	*VF*	*VT*	*VF*	*Pre-drug*	*Isch. -1 min*	*Isch. +10 min*
Control	6/6	4/6	6/6	6/6	158±7	154±10	189±13
0.3 μM	6/6	6/6	5/5	5/5	174±8	132±3	216±14
1 μM	6/6	6/6	6/6	6/6	163±8	151±6	187±6

Table 1 shows that UK-66914 is devoid of actions on QTc, VT and VF and in rat, a species deficient in functional cardiac IK. In contrast, agents which block inward rectifying K+ current (present in rat heart) prolong QTc and inhibit VT and VF in the rat (Rees & Curtis, 1991). Thus, the present data supports the hypothesis that IK blockade and QTc prolongation mediate the antiarrhythmic effects of UK-66914 reported in other species.

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20P THE EFFECTS OF CV1808, AN A_2-ADENOSINE RECEPTOR AGONIST, ON ISCHAEMIC ARRHYTHMIAS IN ANAESTHETIZED PIGS

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Adenosine exerts complex *in vivo* cardiovascular effects mediated by actions at both A_1 and A_2-adenosine receptors. For example, stimulation of A_2 receptors mediates vasodilatation of vascular smooth muscle, release of prostacyclin from endothelial cells, inhibition of platelet aggregation and a depression of leukocyte adhesion. A_1-adenosine receptors, on the other hand, mediate a sinus bradycardia and modification of both the release of noradrenaline from sympathetic nerve endings and the post-junctional increases in cAMP resulting from adrenoceptor stimulation. It is conceivable that all of these effects could contribute to the previous observation that adenosine exerts an antiarrhythmic effect against ischaemic arrhythmias (Wainwright & Parratt, 1988). The aim of the present study was to determine the role of A_2-receptor mediated mechanisms in the prevention of arrhythmias by studying the effects of a selective A_2-receptor agonist CV1808 (2-phenylamino-adenosine; Haleen & Evans, 1985) in a pig model of myocardial ischaemia.

Large white/Landrace cross breed male pigs (28-40 kg) were sedated with azaperone, anaesthetized with chloralose (100 $mgkg_{-1}$ i.v.), and the left anterior descending coronary artery (LAD) prepared for occlusion. Following stabilisation an infusion of CV1808 (1 $\mu gkg^{-1}\ min^{-1}$, n=10) or saline (n=10) was commenced and maintained for the duration of the experiment. Ten minutes after starting the infusion the LAD was occluded and the subsequent arrhythmias were monitored from a Lead II ECG. Standard haemodynamic variables and electrocardiographic (ST-segment and QT interval) measurements were continuously recorded on a computerised data logging system. Blood samples were withdrawn from the coronary sinus at various times before and after starting drug infusion and during coronary occlusion for blood gas determination, prostanoid measurement (by radioimmunoassay) and platelet and leukocyte aggregation studies (by impedence aggregometry).

Prior to coronary occlusion CV1808 did not exert any significant haemodynamic effects, nor did it modify any of the alterations in cardiac function induced by ischaemia. However, coronary sinus oxygen content was increased from 7.0±0.44 ml 100 ml^{-1} prior to starting drug infusion to 8.23±0.45 ml 100 ml^{-1} just prior to occlusion in the CV1808 group, suggesting an increased coronary flow. Ex-vivo aggregation of isolated leukocytes in response to phorbol myristate acetate (PMA) was significantly depressed in samples withdrawn after 10 min of drug infusion (maximum response 4.04±1.33 ohms) compared to control samples (7.83±1.79 ohms, $P<0.05$). Platelet aggregation to collagen, performed in whole blood, remained unchanged (maximum response 15.27±2.74 vs. 14.08±2.52 ohms). Measurement of 6-keto $PGF_{1\alpha}$ in plasma revealed a similar rise in PGI_2 in coronary sinus blood following occlusion in both groups.

Infusion of CV1808 resulted in a decrease in the incidence of ventricular fibrillation (VF) from 100% in controls to 50% ($P=0.0388$) and a reduction in the total number of ventricular premature beats from 281±37 to 161±18 ($P < 0.05$). Furthermore, the ischaemia induced prolongation of the QT-interval was reduced from +50±13 mSEC in controls to +14±12 msec in CV1808 pigs. These results demonstrate an antiarrhythmic effect of CV1808, seen in a dose sufficient to increase coronary flow, and depress leukocyte activation. These mechanisms may therefore make an important contribution to the antiarrhythmic effects observed.

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21P GLIBENCLAMIDE, BUT NOT THE CLASS III COMPOUND E-4031, PROLONGS ISCHAEMIC ACTION POTENTIALS IN GUINEA-PIG PAPILLARY MUSCLE

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Blockade of myocardial K^+ channel subtypes represents a means by which lethal ventricular arrhythmias may be controlled by drugs. While prolongation of action potential duration (APD) and refractoriness in normoxic tissue has been observed with Class III compounds which block time and voltage dependent K^+ currents (Carmeliet, 1985), blockers of ATP-dependent K^+ channels such as glibenclamide (GLIB, Fosset et al., 1988) should prolong the APD in ischaemic tissue (Kodama et al., 1984). The ability of Class III compounds to antagonise arrhythmias arising after acute myocardial ischaemia in vivo is uncertain, since the electrophysiological effects of some Class III compounds are lost during hypoxia (MacKenzie et al., 1990), and their effectiveness during acute ischaemia is reduced in some functional in vitro models (Adams et al., 1990). However, recent reports have shown that a Class III agent, E-4031 (Sawada et al., 1988), decreased the incidence of ischaemic arrhythmias in vivo (Lynch et al., 1990). We have, therefore, studied the in vitro electrophysiological effects of E-4031 under normoxic and ischaemic conditions and compared them to GLIB.

Action potentials (elicited at 1Hz, 2ms pulse width) were recorded by microelectrode from guinea-pig (350-500g) papillary muscles at 36.5°C under normoxic (Krebs gassed by 95% O_2/5% CO_2) or ischaemic (glucose-free Krebs at pH 6.9, K^+ 9.2mM, gassed by 55% O_2/ 40% N_2/5% CO_2) conditions. Action potential amplitude (APA) and duration (APD) were determined. E-4031 (1-60μM) prolonged normoxic APD_{90} in a concentration-dependent manner (control=95 ± 29.3ms; 10μM E-4031=124 ± 31.9ms, n=7, $p<0.01$) while GLIB had little effect (control=129 ± 5.8ms; 10μM GLIB=127 ± 6.6, n=6, insignificant). APA and APD decreased under ischaemic conditions before they reached a steady-state value. After 20 min ischaemia, APD_{90} was reduced by 73 ± 10.1%, n=9. E-4031 (10μM for 7min) then increased the APD_{90} by 28% (20min ischaemia=30 ± 9.6ms; 10μM E-4031=37 ± 9.3ms, n=7, insignificant) whereas GLIB (10μM for 7min) increased APD_{90} by 132% (20min ischaemia=33 ± 14.6; 10μM GLIB=67 ± 31.5ms, n=9, $p<0.05$). GLIB time-dependently increased the APD_{90} (10μM GLIB for 30min=102 ± 11.1ms, n=6, $p<0.001$).

We have confirmed that E-4031 has Class III activity under normoxic conditions. Although E-4031 antagonised ischaemic arrhythmias in vivo (Lynch et al., 1990), it did not significantly prolong ischaemic APD in vitro, unlike the ATP-dependent K^+ channel blocker GLIB. Some additional mechanism may contribute to the action of E-4031 against ischaemic arrhythmias in vivo.

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22P MODIFICATION BY HYPERKALAEMIA OF THE ELECTROPHYSIOLOGICAL EFFECTS OF ISOPRENALINE ON GUINEA-PIG VENTRICULAR MYOCYTES

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One of the early consequences of myocardial ischaemia is an elevation of the extracellular concentration of K^+ and release of catecholamines. The aim of this study was to determine whether hyperkalaemia modified the electrophysiological effects of isoprenaline on guinea pig ventricular myocytes. Action potentials were measured at a stimulation frequency of 1Hz using current clamp techniques in myocytes isolated by enzymatic dispersion, and superfused with a physiological salt solution at 34°C.

Isoprenaline (0.1 - 100 nM) caused a concentration dependent shortening of the action potential duration measured at both 50% (APD_{50}) and 90% (APD_{90}) of repolarisation (Figure 1, left hand panel) without changing the resting membrane potential or the upstroke of the action potential. Subsequent experiments were carried out with 10 nM isoprenaline. Elevating the extracellular concentration of K^+ from 5.4 (normal) to 8 or 10 mM caused significant and concentration dependent reductions in resting membrane potential, action potential amplitude, APD_{50} and APD_{90}. For example, 10 mM K^+ reduced the resting membrane potential from -87 ± 2 to -71 ± 2 mV, action potential amplitude from 123 ± 1 to 110 ± 3 mV and APD_{50} and APD_{90} from 131 ± 10 to 106 ± 13 and 167 ± 10 to 130 ± 13 ms respectively (n = 5). In the presence of an elevated K^+ concentration, the ability of isoprenaline to abbreviate the action potential was either attenuated (with 8 mM K^+) or lost (with 10 mM K^+) (Figure 1, right hand panel). Isoprenaline did not affect the other action potential variables in the presence of hyperkalaemia.

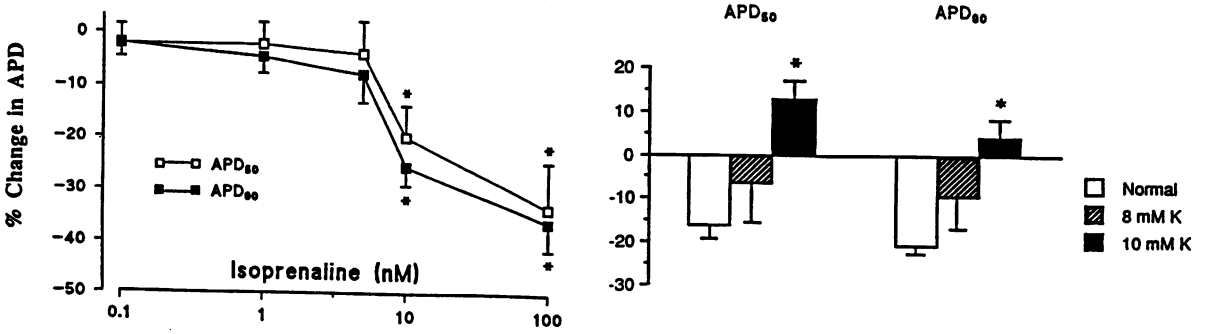

Figure 1. The effects of isoprenaline on action potential duration in normal K^+ (left hand panel) and the modification of these effects by hyperkalaemia (right hand panel). n = 5-10. *$P<0.05$ significantly different from appropriate control.

It is concluded that elevation of the extracellular K^+ concentration modifies the cardiac electrophysiological effects of isoprenaline such that its ability to shorten the normal action potential is lost.

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23P HALOTHANE ACTIONS ON THE CYTOSOLIC CALCIUM TRANSIENT DURING ACTION POTENTIALS IN VENTRICULAR CELLS ISOLATED FROM GUINEA-PIG HEART

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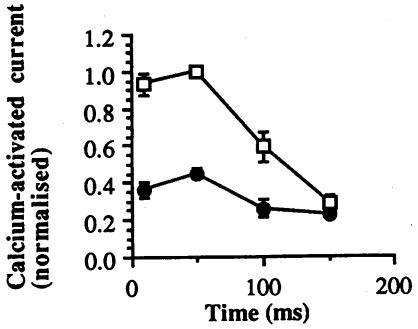
Halothane has been shown to reduce calcium currents in isolated guinea-pig ventricular cells, and this action is thought to contribute to its negative inotropic effect; evidence has also been presented for additional actions of halothane on calcium handling by the sarcoplasmic reticulum (Terrar & Victory, 1988). The aim of the present work was to further investigate the possible action of halothane on the cytosolic calcium transient by using calcium-activated currents during interrupted action potentials as an indirect indicator of cytosolic calcium (see Terrar & White, 1989).

Single cells were isolated from guinea-pig ventricular muscle. Action potentials were interrupted by application of 300 ms voltage clamp steps to -40 mV in order to evoke the calcium-activated current. The contributions of other currents are thought to be negligible under these conditions (Terrar & White, 1989). Voltage-clamp steps were applied 10, 50, 100, and 150 ms after the upstroke, and the envelope of the amplitudes of calcium-activated currents indicated the time course of cytosolic calcium (see Figure 1). Temperature was 36℃, stimulation rate was 1 Hz and eight full action potentials preceded each interruption to ensure that filling of the sarcoplasmic reticulum stores with calcium had reached a steady state.



Calcium-activated currents, normalised to those for action potentials interrupted at 50 ms, are shown as open squares in Figure 1 (bars show S.E.M.). Halothane at 2% (1.47 mM) caused a marked suppression of the calcium-activated currents (Figure 1, filled circles). These effects were significant ($P < 0.01$, n=9) at 10, 50 and 100 ms after the upstroke. The effects of halothane were fully reversible on washout of the drug. Halothane at 1% (0.60 mM) reduced the calcium activated current early in the action potential ($P < 0.05$ at 10 ms, n=9), while there was no change or an increase in the calcium-activated current at later times in the action potential. When cells were exposed to 10 mM caffeine to interfere with calcium handling by the sarcoplasmic reticulum, the envelope of calcium-activated currents was prolonged. Under these conditions, halothane at 2% significantly reduced the current at 10, 50, 100 and 150 ms ($P < 0.01$, n=13).

These observations are consistent with an action of halothane on the cytosolic calcium transient. Effects of halothane on calcium influx through calcium channels and on calcium handling by the sarcoplasmic reticulum stores may contribute to these effects.
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24P ZAPRINAST POTENTIATES ADENOSINE-INDUCED BRADYCARDIA AT DOSES THAT DO NOT ALTER HAEMODYNAMIC RESPONSES TO SODIUM NITROPRUSSIDE OR SALBUTAMOL

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It has been observed previously that the cyclic GMP-specific phosphodiesterase (PDE V) inhibitor zaprinast increased arterial blood pressure (Holbrook & Coker, 1991). The present experiments were designed to investigate the effects of zaprinast and the non-selective PDE inhibitor isobutylmethylxanthine (IBMX) on haemodynamic responses mediated via cyclic GMP and cyclic AMP using sodium nitroprusside and salbutamol respectively. Responses to adenosine were also studied.

Male Wistar rats were anaesthetized with sodium pentobarbitone 60 mg kg^{-1} i.p. A carotid artery was cannulated for blood pressure measurement and a femoral vein for drug administration. Dose response curves to sodium nitroprusside, salbutamol or adenosine were constructed before and after administration of increasing doses of zaprinast or IBMX. Bolus doses of agonist were given at 5 min intervals and at least 10 min was allowed between dose response curves. After obtaining 3 control curves the PDE inhibitor was administered 10 min prior to the next dose response curve. Only one agonist and one PDE inhibitor were examined in each rat and there were 5 rats in each group.

Administration of zaprinast 0.3, 1 or 3 mg kg^{-1} had no sustained effect on blood pressure itself and did not significantly alter the depressor responses to sodium nitroprusside. For example, in control conditions, 8 μg kg^{-1} sodium nitroprusside caused a 52 ± 5% decrease in diastolic blood pressure whereas the corresponding change after the highest dose of zaprinast was 59 ± 2%. Similarly, zaprinast also failed to alter the depressor responses to salbutamol and adenosine. The reduction in heart rate of 30 ± 2 beats min^{-1} induced by 200 μg kg^{-1} adenosine was increased to 45 ±7, 59 ± 5 and 65 ± 5 beats min^{-1} after 0.3, 1 and 3 mg kg^{-1} zaprinast, respectively ($P<0.05$, highest dose cf. control). IBMX 0.1, 0.3 and 1 mg kg^{-1} did not alter significantly the depressor responses to any of the agonists, or the bradycardia induced by adenosine.

These results indicate that at the doses used, neither zaprinast nor IBMX altered cyclic GMP- or cyclic AMP-mediated depressor responses. This may suggest that these doses were insufficient to increase cyclic nucleotides significantly *in vivo*. The results also suggest that the ability of zaprinast to potentiate adenosine-induced bradycardia is unlikely to be related to effects on cyclic nucleotides and that further experiments are necessary to determine whether this effect is dependent on inhibition of adenosine uptake or some other mechanism of action.

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25P THE NOVEL NEUROPROTECTIVE AGENT, RS-87476, POTENTLY INHIBITS SODIUM CURRENTS IN MOUSE NEUROBLASTOMA

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RS-87476 (*piperazine 1-[diphenyl-methyl]-4-[(2-(4-methyl-phenyl)-5-methyl imidazol-4-yl)methyl] trihydrochloride*) displays marked neuroprotective activity in a variety of animal models of CNS ischaemia: rat four-vessel occlusion (Alps *et al.*, 1990), gerbil bilateral carotid artery occlusion (Alps *et al.*, unpublished) and cat middle cerebral artery (Kucharczyk *et al.*, 1991). At the mechanistic level there is good evidence that the compound interacts with the voltage-dependent sodium channel: RS-87476 inhibits veratrine-induced contracture of chick cardiac myocytes (IC_{50} < 2 µM; Patmore *et al.*, this meeting) and potently displaces *[³H]*-batrachotoxin in synaptosomes (IC_{50} = 119 nM; B. Kenny *et al.*, unpublished). We have, therefore, examined the actions of RS-87476 on sodium currents in the mouse neuroblastoma clone, N1E-115, in an effort to establish the functional effects of this compound directly at the sodium channel.

Sodium currents (I_{Na}) were recorded from N1E-115 cells using conventional whole cell patch-clamp techniques (Hamill *et al.*, 1981). Extra- and intracellular solutions were similar to those described by Huguenard *et al.* (1988). Experiments were conducted at room temperature (18-22 °C).

In the first series of experiments the effects of RS-87476 were examined on the activation of I_{Na}. Current/voltage relationships were obtained before and 10 min after RS-87476 application. A time-matched control group, in which no drug was applied, was used to test for any drug-independent changes in I_{Na} activation. The effects of RS-87476 were examined on the Boltzmann parameters for half-maximum activation potential (V_H), slope of the activation curve (k) and maximum conductance (g_{MAX}) (see, for example, Ginsborg *et al.*, 1991). The results, based on a total of 14 cells voltage-clamped at -80 mV, are shown in the Table (pooled pretreatment values: V_H -14.5 ± 1.1 mV; k 7.2 ± 0.3 mV; g_{MAX} 42.6 ± 5.1 nS). The data show that g_{MAX} was the only parameter of activation to be modified by RS-87476.

EFFECT OF RS-87476 ON PARAMETERS OF ACTIVATION

	n	ΔV_H (mV)	Δk (mV)	Δg_{MAX} (%)
Control	5	-2.9 ± 1.2	-0.02 ± 0.48	4.8 ± 17
RS-87476 (1µM)	5	-0.3 ± 1.8	1.19 ± 0.66	-44.0 ± 6.4 *
RS-87476 (3µM)	4	0.7 ± 1.5	1.84 ± 0.83	-70.0 ± 9.7 *

[Data are means ± sem] * *paired 2-tailed t-test: P < 0.05*

In the second series of experiments the actions of RS-87476 were examined on a test current evoked 10s after a long (20s) conditioning depolarization (V_{COND}) to -60 , -30 or 0 mV (holding potential -100 mV; test I_{Na} evoked by 20 ms step to 0 mV). In untreated cells the amplitude of the test I_{Na} was unchanged from the preconditioning control I_{Na} (n = 2 cells). However, in the presence of 1µM RS-87476 the test I_{Na} was reduced by 14 ± 6%, 54 ± 15% or 60 ± 19% of its control amplitude (V_{COND} -60, -30 or 0 mV, respectively). Moreover, I_{Na} recovered exponentially to control levels during subsequent test depolarizations.

These findings demonstrate that RS-87476 is a potent voltage-dependent inhibitor of I_{Na} ($IC_{50} \geq$ 1µM) and that the compound may preferentially interact with the inactivated state of the sodium channel, thereby reducing the number of channels available for activation. Whether inhibition of I_{Na} underlies the neuroprotective mechanism of RS-87476 remains to be established.

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26P DRUG-SENSITIVITY OF SODIUM UPTAKE IN HUMAN PLACENTAL BRUSH BORDER MEMBRANE VESICLES

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Defective regulation of chloride (Cl) channels in epithelial tissue is now known to be associated with the basic defect in cystic fibrosis. There is also evidence of abnormal sodium (Na) permeability in CF epithelia (Boucher et al., 1988). We have previously reported on Cl transport in brush border membrane vesicles (BBMV) prepared from human term placenta (Faller et al., 1990). The present study concerns Na uptake into human placental BBMV.

BBMV were prepared by a magnesium precipitation technique. Purity of the vesicles was assessed by marker enzyme assays and by electron microscopy. Vesicle size was determined using a BD FAC Star Plus flow cytometer and appropriate marker beads. Na uptake was measured in the presence of an outwardly directed Na gradient using a modification of the method of Garty et al. (1983). Using this system, we have found a time-dependent accumulation of ^{22}Na within the vesicles. ^{22}Na uptake was linear for approx 10 mins and reached equilibrium between 20-40 mins. Maximum uptake was calculated to be 0.018 ± 0.002 pmol Na/mg protein (mean ± s.e. mean; n=4). The activation energy of the uptake process was calculated by measuring ^{22}Na uptake over 2 mins at 4, 25 and 37°C. This was found to be 4.81 ± 0.68 kcal/mol. Neither ouabain or bumetanide had any significant effects on Na uptake at concentrations up to 100 μM. A dose-dependent inhibition of Na uptake by amiloride was demonstrated with an IC_{50} of 3.08 ± 0.74 μM. No effect on Na uptake was demonstrated at 1 and 10 μM concentrations of 4-acetamido-4'-isothiocyano-stilbene-2,2'-disulphonic acid (SITS) but significant inhibition was demonstrated at 100 and 1000 μM. Linoleic acid, which we have previously shown to inhibit Cl uptake into BBMV, did not affect Na uptake at concentrations up to 100 μM.

These results indicate that under the conditions employed in our assay, Na uptake into BBMV was channel mediated with an activation energy similar to that of ionic diffusion in free solution. Na transport was inhibited by amiloride which is an established inhibitor of Na channels. Na-K-ATP'ase activity was not involved as ouabain did not inhibit the Na transport. The Na-K-Cl cotransporter was also not involved as bumetanide had no effect. This system may be useful in investigation of Na transport defects in CF.

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27P INHIBITION OF BINDING TO TACHYKININ NK_1 RECEPTOR SITES IN RAT, GUINEA-PIG AND HUMAN BRAIN BY THE NON-PEPTIDE SUBSTANCE P ANTAGONIST (±)CP-96,345

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The recently described non-peptide NK_1 tachykinin receptor antagonist CP-96,345 [(2S,3S)-cis-2-(diphenylmethyl)-N-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine] demonstrates marked species variation in its affinity for brain NK_1 receptor binding sites. Thus, CP-96,345 inhibits binding of [^{3}H]-substance P to NK_1 sites in bovine caudate nucleus and rat forebrain membranes with IC_{50} values of 3.4nM and 240nM, respectively (Snider at al., 1991), whilst [^{3}H]-CP-96,345 labels NK_1 sites in guinea pig striatal membranes with a Kd value of 0.22nM (McLean et al., 1991). In the present study, we have compared the ability of racemic (±)CP-96,345 [a mixture of the two enantiomers (2S,3S) and (2R,3R)], the naturally occurring tachykinins substance P (SP), neurokinin A (NKA) and neurokinin B (NKB), and the peptide based substance P antagonist [D-Arg^1,D-Trp^{7,9},Leu^{11}]SP (spantide) to inhibit binding of [^{125}I]-Bolton Hunter labelled substance P ([^{125}I]-BHSP) to NK_1 binding sites in homogenates of rat striatum, guinea pig striatum and post-mortem human caudate nucleus.

Binding assays were performed according to the method of Lee et al., (1983), with minor modifications. Membranes (20-100µg protein/tube) were incubated (60min at room temperature) with [^{125}I]-BHSP (0.1nM) in a final volume of 300µl in the presence of 10^{-11}-10^{-5}M displacer, and then rapidly filtered through Whatman GF/C filters using a Brandel Cell Harvester. Substance P (10^{-6}M) was used to define non-specific binding. Data from 3-5 individual displacement curves were analysed using an iterative curve fitting programme on RS1 and are expressed as mean pIC_{50} ± SEM (mean Hill slope ± SEM).

Table 1 Inhibition of [^{125}I]-BHSP binding in rat, guinea pig and human brain

	SP	NKA	NKB	(±)CP-96,345	Spantide
Guinea pig striatum	9.99±0.09 (0.87±0.08)	7.78±0.07 (0.89±0.08)	7.28±0.07 (0.92±0.7)	8.20±0.24 (0.97±0.05)	6.12±0.05 (1.12±0.07)
Human caudate nucleus	9.97±0.04 (0.97±0.12)	7.78±0.05 (0.86±0.03)	6.89±0.06 (0.92±0.08)	8.72±0.03 (0.99±0.06)	6.59±0.11 (0.92±0.05)
Rat striatum	10.12±0.02 (0.89±0.05)	7.90±0.14 (0.81±0.05)	7.15±0.29 (0.82±0.05)	6.91±0.13 (0.77±0.06)	6.09±0.09 (0.85±0.03)

These results indicate that the non-peptide SP antagonist (±)CP-96,345 possesses a lower affinity for central NK_1 binding sites in rat striatum as compared with guinea pig striatum and human caudate nucleus. In contrast, SP, NKA, NKB and spantide each possess similar affinities for central NK_1 sites in the above three tissues. Further experiments are necessary in order to determine whether the differences in affinity observed with (±)CP-96,345 relate to species differences or multiple NK_1 binding sites.

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28P ACTIVITY OF A NON-PEPTIDE NK_1 ANTAGONIST, RACEMIC CP-96,345, AT NK_1 RECEPTORS IN THE RAT AND MOUSE *IN VIVO*

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The pharmacology of a potent, selective, non-peptide NK_1 receptor antagonist, CP-96,345 has been described (Snider et al., 1991). In the present study the racemic form of this compound (cis-2-(diphenylmethyl)-N-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine; Beresford et al., this meeting) has been evaluated for NK_1 receptor antagonist activity *in vivo* against (a) paw oedema in the rat induced by intraplantar co-injection of the NK_1 agonist, GR73632, and CGRP (Beresford et al., 1991) (b) caudally-directed biting/scratching (CDBS) in the rat after intrathecal injection of GR73632, and (c) CDBS in the mouse after intrathecal injection of substance P methylester (SPOMe). The effects of racemic CP-96,345 have been compared with the selective, peptide NK_1 receptor antagonist GR82334 (Hagan et al., 1991).

Male RH, Glaxo-bred rats (75-120g) were injected (100µl) by the intraplantar route with GR73632 (0.03 to 1 nmol) in the presence of a subthreshold dose of CGRP (0.1 nmol), and the resulting paw oedema measured by use of a plethysmometer. Pretreatment (30min) with GR82334 (0.07 or 2.2 µmol/kg sc; n=5) or racemic CP-96,345 (24 µmol/kg sc; n=5) produced a rightward shift in the dose-response curve to GR73632/CGRP; dose-ratios (95% confidence limit) were 5.7 (3-10), 893 (271-7692) and 22.3 (7-64), respectively. Increasing the pretreatment time with racemic CP-96,345 to 60 or 90min produced dose-ratios of 26.0 (10-77) and 6.5 (2-19), respectively. Racemic CP-96,345 (24 µmol/kg sc; 30min pretreatment) had no effect on paw oedema induced by intraplantar injection of bradykinin (1 to 100 nmol; n=10) or 5HT (0.1 to 10 nmol; n=5).

In the rat (male RH, Glaxo-bred, 50-75g), intrathecal injection (10µl) of GR73632 produced CDBS (ED_{50} 1.2 (0.2-2) pmol, n=6). Intrathecal pretreatment (5-min; n=6) with 1 nmol GR82334 produced a 9.0 (4-22)-fold shift in the dose-response curve to GR73632. However, intrathecal pretreatment (5min; n=6) with racemic CP-96,345 (1 or 50 nmol) produced smaller shifts; dose-ratios were 1.5 (0.4-9) and 5.0 (2-9), respectively, Subcutaneous administration (24µmol/kg; 30min pretreatment; n=6) was ineffective; dose-ratio 0.5 (0.3-1). In the mouse (male CRH, Glaxo-bred, 17-22g) intrathecal injection (5µl) of SPOMe produced CDBS (ED_{50} 2.9 (2-7) pmol, n=6). Intrathecal administration of GR82334 (0.7 nmol; co-injection or 5min pretreatment; n=6) produced large shifts in the SPOMe dose-response curve; dose-ratios were 25.8 (12-77) and 19.6 (6-83), respectively. In comparison, intrathecal injection of racemic CP-96,345 (50 nmol; co-injection, 5min or 15min pretreatment; n=6) produced small shifts; dose-ratio were 2.5 (1-5), 4.7 (2-11) and 3.7 (2-10), respectively. Subcutaneous administration of racemic CP-96,345 (73 µmol/kg; 30min pretreatment) failed to antagonise the response to SPOMe (dose-ratio 1.1 (0.5-2); n=6), whereas intravenous administration (7.3 µmol/kg, 10min pretreatment; n=6) produced only a small shift; dose-ratio 2.8 (1-6).

The results indicate that GR82334 is more potent than racemic CP-96,345 as an antagonist of both peripherally-mediated and centrally-mediated NK_1 responses in the rat and mouse *in vivo*. This is in contrast to published data *in vitro* where GR82334 is a weaker NK_1 antagonist than the non-peptide compound (Hagan et al., 1991; Snider et al., 1991). The reason for this is unkown, but may relate to the existence of species variants of the NK_1 receptor (Beresford et al., this meeting).

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29P NEUROPROTECTIVE EFFECT OF THE κ-AGONIST, CI-977, IN THE GERBIL MODEL OF GLOBAL ISCHAEMIA

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Recent evidence suggests that κ-agonists may be effective in reducing the neurological damage occuring as a result of cerebral ischaemia in animals (Tang, 1985; Hall & Pazara, 1988). We have investigated this further by testing the effectiveness of the potent and selective κ-agonist, CI-977 (Hunter *et al.*, 1990), to protect against neuronal degeneration produced by transient global ischaemia in the gerbil.

Under forane anaesthesia, female Mongolian gerbils (60-80g) were subjected to 7 min forebrain ischaemia by occluding both carotid arteries. CI-977 was administered immediately before and at 4 h after ischaemic insult. The NMDA-antagonist, MK-801 (Dizocilpine) was administered immediately before the ischaemic insult. In experiments investigating the actions of the opioid antagonist naltrexone, this drug was co-administered with either CI-977 or MK-801. Seven days later animals were sacrificed and measurements of neuronal damage were summed for both hippocampi (CA1/CA2) in cresyl fast violet coronal sections (20 μm) corresponding to 1.6 mm caudal to bregma using an image analyser.

Table 1 Effect of CI-977 & MK-801 on the degree of ischaemia-induced hippocampal degeneration

			Dose of CI-977 (mg kg^{-1} sc)			Dose of MK-801 (mg kg^{-1} sc)		
	Sham	Control	0.3	1	3	1	3	10
Area (μm^2)	374.6	108.3	175.0*	267.5*	323.1*	171.0	248.1*	362.3*
mean ± SEM	5.8	11.0	22.1	25.2	15.8	21.9	22.8	14.3
(n)	(25)	(25)	(17)	(16)	(21)	(7)	(16)	(8)

* P < 0.05 (compared to controls; Dunnetts t-test)

In control animals, marked degeneration of hippocampal CA1/CA2 pyramidal neurones was seen. CI-977 and MK-801 dose-dependently decreased the area of neuronal damage (Table 1). Naltrexone (10 mg kg^{-1} sc) completely antagonised the neuroprotective effect of CI-977 (1 mg kg^{-1} sc), without affecting the neuroprotective effect of MK-801 (3 mg kg^{-1} sc), and itself having no effect on neuronal damage.

These results indicate that CI-977 is an effective neuroprotective agent in the gerbil model of transient global ischaemia. The ability of naltrexone to antagonise this protective effect, is consistent with CI-977 having an action at the κ-opioid receptor. The neuroprotective action may in part be due to an interaction between the κ-receptor and the NMDA receptor, since CI-977 is anticonvulsant against NMDLA-evoked seizures in mice (Singh *et al.*, 1990), and inhibits potassium evoked glutamate release from cortical slices (Lambert *et al.*, 1991).

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30P THE ROLE OF TUMOUR NECROSIS FACTOR AND THE LEUKOCYTE ADHESION MOLECULE CR3 IN ENDOTOXIC SHOCK

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Endotoxaemia resulting from Gram negative infection often leads to septic shock and death. Endotoxin (lipopolysaccharide; LPS) stimulates the release from leukocytes of cytokines such as tumour-necrosis factor (TNF) which in turn induce the expression of adhesion molecules for leukocytes such as ELAM on the surface of vascular endothelial cells. Following the adherence of leukocytes to the endothelium the leukocyte integrins (LFA1, CR3 and p150,95) are important in the movement of the cells into the tissues. The interactions of leukocytes with the blood vessel wall lead to changes in permeability and reactivity which contribute to the systemic hypotension, tissue hypoxia and eventual multi-organ failure seen in septic shock. We have now investigated the effects of monoclonal antibodies (Mabs) to TNF and CR3 on LPS-induced mortality in mice.

Groups of 10 Balb/c mice were pretreated intravenously (0.1ml) with a Mab to murine TNF (TN3, 20mg/kg; Sheehan et al., 1989), a Mab to murine CR3 (5C6, 20mg/kg; Rosen et al., 1989), a control antibody (L2, 20mg/kg) or saline. One hour later, the mice were injected intravenously (0.1 ml)with 40mg/kg LPS (Sigma, serotype 0111B4) and observed for 72 hours. In some experiments, total and differential leukocyte counts were determined on blood samples taken by cardiac puncture at different time points after LPS injection.

In groups of animals pretreated with saline or L2, LPS induced a mortality at 24h of 34.8% ± 7.5 and 47.1% ± 11.5 respectively. This effect was significantly reduced to 5.7% ± 3.7 ($p<0.01$) by TN3 and enhanced to 75.3% ± 6.7 ($p<0.001$) by 5C6. Mortality in control or 5C6-treated animals was 90% or greater at 72h whereas less than 10% of TN3-treated animals had died at this time. Combinations of TN3 and 5C6 gave a similar protection to TN3 alone. In all groups of animals a profound leukopaenia was observed following LPS injection. At 2h, however, TN3-treated animals had greater numbers of circulating neutrophils than the other groups.

These results confirm the observation that TN3 protects against LPS-induced mortality in mice (Sheehan et al., 1989). This indicates that TNF is a prime mediator in the pathogenesis of septic shock. It is possible that TN3 protection is through a down-regulation of the leukocyte-endothelium interaction. Antibodies to CD18, the common beta chain of the leukocyte integrins, are more effective in blocking transendothelial migration of neutrophils than in blocking initial adhesion (Lawrence, et al., 1990). It is possible, therefore, that 5C6 exacerbates endotoxic shock by preventing neutrophils from moving through the blood vessel wall and thereby enhancing their interactions with the vascular endothelium. In models of extra vascular inflammation, however, 5C6 has a beneficial effect (Rosen et al. 1989).

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31P HOMOCYSTEINE-INDUCED ENDOTHELIAL BARRIER DYSFUNCTION ASSESSED BY AN *IN VITRO* MODEL

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In atherosclerosis, endothelial barrier dysfunction leads to increased entry of cholesterol-rich low-density lipoproteins into the arterial wall. An elevated circulating level of homocysteine is a known risk factor in the development of atherosclerosis (McCully, 1969). In this study we wished to determine, using an in vitro model (Gudgeon & Martin, 1989), if homocysteine induced endothelial barrier dysfunction. This being so, we investigated the involvement of oxygen-derived free radicals by comparing the effects of homocysteine to those of the hypoxanthine-xanthine oxidase system.

Endothelial barrier function was assessed by measuring, over a 90min incubation period, the transfer of trypan blue-labelled albumin (4%) across monolayers of bovine aortic endothelial cells (BAEC) grown on Costar Transwell membranes (3μm pore size).

Unstimulated transfer of albumin across BAEC was 6.9±0.8% (n=30). Neither hypoxanthine (0.2mM) nor xanthine oxidase ($20mUml^{-1}$) had any effect on albumin transfer, but a combination of these stimulated transfer to 18.2±1.8% (n=6). This increase in albumin transfer was not affected by superoxide dismutase ($30Uml^{-1}$) but was abolished by catalase ($3Uml^{-1}$). Hydrogen peroxide (0.1-30mM) itself induced a concentration-dependent increase in albumin transfer: the maximum obtained was 33.5±3.9% (n=6). This increase in albumin transfer was inhibited by catalase ($300-3000Uml^{-1}$). Homocysteine (1.5mM) did not affect albumin transfer, but in the presence of copper sulphate (50μM), which catalyses its oxidation, transfer was increased to 20.8±2.0% (n=6). This increase in albumin transfer was abolished in the presence of catalase ($100Uml^{-1}$).

The data show that oxidation of homocysteine can induce endothelial barrier dysfunction in a manner similar to the hypoxanthine-xanthine oxidase free radical generating system. The effectiveness of catalase suggests that the endothelial barrier dysfunction is a consequence of the generation of hydrogen peroxide. It is possible that endothelial barrier dysfunction contributes to the development of atherosclerosis seen in homocystinuria.

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32P ENDOTHELIAL FUNCTION AND NORADRENALINE SENSITIVITY IN MESENTERIC RESISTANCE ARTERIES OF RATS WITH STREPTOZOTOCIN-INDUCED DIABETES

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Abnormal endothelial function has been implicated in diabetic vascular disease and a number of reports have suggested this is associated with reduced production of endothelium derived relaxing factor (EDRF)[1,2]. Previous studies have focused on conduit arteries; in this study we have investigated resistance artery function in rats with streptozotocin-induced diabetes (STZ).

Diabetes was induced in female CSE rats (n=12, 250g) by i.p. injection of streptozotocin (55mg/kg). After 5-6 weeks the animals were killed by cervical dislocation and mesenteric resistance arteries (i.d.STZ 284 ± 8.5 μm; CTR 264 ± 7.2 μm) dissected and mounted on a small vessel myograph. Noradrenaline (NA) concentration responses were determined ($5x10^{-8}$M - $5x10^{-6}$M). Endothelium-dependent relaxation was then determined by acetylcholine (ACh)-induced relaxation (10^{-9}M - 10^{-5}M) in arteries pre-contracted with NA (3μM). This procedure was then repeated in the presence of 10μM indomethacin (IND), and again in 10μM IND + 1mM LNMMA (N^G-monomethyl-L-Arginine). Endothelium-independent relaxation was assessed by sodium nitroprusside (SNP)-induced relaxation, (10^{-9}M-10^{-5}M). Blood glucose levels were 47.7 ± 7.7 mM (STZ) vs. 7.3 ± 1.1 mM (CTR).

The STZ arteries demonstrated enhanced sensitivity to noradrenaline, and impaired ACh-induced relaxation (CTR, EC_{50}=4.74 ± 0.46 x 10^{-8} M, n=20 arteries; STZ, EC_{50}=4.83±0.47x10^{-7}M, n=18, $p<0.001$). In the presence of indomethacin, ACh-induced relaxation remained significantly different between STZ and CTR vessels. Addition of LNMMA led to much greater inhibition in STZ compared to CTR (ACh 10^{-5}M, STZ 86.55±3.33% inhibition; CTR 51.06±7.07 %, $p<0.001$). This incomplete inhibition of relaxation in CTR was likely to be the result of greater EDRF release than in STZ. This was confirmed by the use of LNAME (10^{-4}M), a more potent inhibitor of EDRF, which led to greater inhibition of ACh-induced relaxation in further CTR experiments (ACh 10^{-5}M, 74.50±5.16 % inhibition, n=5; $p<0.05$). Relaxation to SNP (endothelium-independent) was similar in STZ and CTR.

Enhanced sensitivity to NA and impaired endothelium-dependent relaxation may contribute significantly to abnormal vascular function in the resistance arteries of rats with streptozotocin-induced diabetes.

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33P BLOCK OF ATP-REGULATED K^+ CHANNELS BY α_2-ADRENOCEPTOR ANTAGONISTS IN INSULIN-SECRETING CELLS

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Certain α-adrenoceptor antagonists, such as phentolamine (α_1 and α_2) and efaroxan (α_2) are known to stimulate insulin secretion by a mechanism that does not solely involve binding to α-adrenoceptors (Plant & Henquin, 1990; Chan & Morgan, 1990; Dunne, 1991). In the present study we have measured insulin secretion and $^{86}Rb^+$ efflux in mammalian β-cells and applied the patch-clamp technique to RINm5F cells in order to further investigate the actions of efaroxan on stimulus-secretion coupling.

In the absence of adrenoceptor agonists, efaroxan (1 - 100 μM) significantly enhanced glucose-induced secretion over the range 3 - 12 mM, but was found to be ineffective in the presence of 20 mM glucose. In perifused islets the secretory response to efaroxan rapid, sustained and followed a monophasic time course. It was not accompanied by any significant change in islet cAMP levels. Suppression of insulin secretion by the potassium channel openers diazoxide (250 μM) and pinacidil (250 μM), was partially alleviated by efaroxan (20 - 100 μM) and was associated with a marked inhibition of the diazoxide-induced increase in $^{86}Rb^+$ efflux. Patch-clamp experiments carried out using outside-out patches and permeabilized or open-cells indicated that when efaroxan (1 - 100 μM) was added to either the inside or the outside of the plasma membrane, a concentration-dependent inhibition of ATP-sensitive K^+ channels resulted. The actions of efaroxan were fully reversible and not dependent upon the presence of intracellular ATP. The K_i value corresponding to 50% inhibition of channels was approximately 12 μM and the Hill coefficient found to be approximately 1.

In conclusion these data suggest that the actions of efaroxan on insulin-secreting cells are associated with the modulation of ATP-sensitive K^+ channels. Efaroxan-induced closure of these channels results in enhanced insulin secretion from intact islets of Langerhans.

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34P DENDROTOXIN-LIKE ACTIVITY ISOLATED FROM SEA-ANEMONES

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Many sea anemones are known to contain neurotoxins that act on Na^+ channels. With the recent discovery of toxins directed against K^+ channels from a variety of animals (Harvey, 1991), we examined extracts from two Caribbean sea anemones for possible K^+ channel toxins. Extracts and fractions were screened for the ability to facilitate acetylcholine release using the chick biventer cervicis nerve-muscle preparation and for competition with ^{125}I-labelled dendrotoxin-I to rat brain synaptosomal membranes (Harvey *et al.*, 1991).

Aqueous extracts were prepared from the sea anemones *Stichodactyla helianthus* and *Bunodosoma granulifera*. They were found to increase the responses of chick biventer cervicis preparations to indirect stimulation. Extracts were fractionated on a Sephadex G-50 gel filtration column and five major fractions were collected. In both cases, one fraction contained most of the facilitatory activity and also reduced binding of ^{125}I-dendrotoxin-I. These fractions contained polypeptides and small proteins. The active fractions from the two sea anemones were separated by ion exchange chromatography using a TSK SP-5PW HPLC column. Eight peaks were collected from the *Bunodosoma granulifera* G-50 fraction and ten from the *Stichodactyla helianthus* fraction. The last, most basic, peak from *Bunodosoma granulifera* competed with dendrotoxin binding and augmented responses of chick biventer cervicis preparations to indirect stimulation, without affecting postjunctional sensitivity. Three peaks from *Stichodactyla helianthus* (Sh SP6, Sh SP7, and Sh SP7A) competed with dendrotoxin binding, and two of them (Sh SP7 and SP7A) also selectively augmented responses to indirect stimulation. Preliminary amino acid analysis revealed that Sh SP6 is homologous to Kunitz protease inhibitors, including dendrotoxins, and Sh SP6 was demonstrated to inhibit the activity of trypsin with a K_i of 8nM.

The results indicate that there are dendrotoxin-like facilitatory toxins in the two sea anemones. Further work will establish if they act like dendrotoxins on neuronal K^+ channels (Harvey & Anderson, 1985).

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35P COMPARATIVE SECOND MESSENGER BINDING AND *IN SITU* HYBRIDIZATION STUDIES OF THE MEDIAL TEMPORAL LOBE IN ALZHEIMER'S DISEASE

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Abnormalities in a number of neurotransmitter systems have been found in Alzheimer's disease, notably in the cholinergic (Perry *et al*, 1977), glutamatergic (Greenamyre *et al*, 1987), and serotoninergic (Cross *et al*, 1988) systems. In the light of the knowledge that drug therapy targeted at these neurotransmitter systems has had limited efficacy, it might be more useful to target those components of neuronal function which are common to many of the main neurotransmitter systems and their functional receptors, such as second messenger enzymes. Abnormalities of second messenger systems such as decreased levels of protein kinase C in the brain in general (Cole *et al*, 1988) and reductions in forskolin binding to activated adenylyl cyclase in the frontal and temporal cortex (Dewar *et al*, 1990) have been found in Alzheimer's disease brain.

The region of the medial temporal lobe has proved to be an area of intense neuropathological interest in Alzheimer's disease in recent years (Pearson *et al*, 1985). For this reason, the temporal lobe from seven patients confirmed neuropathologically to have had Alzheimer's disease and seven age- and sex-matched controls have been studied here. In this communication we describe the results of attempts to map the location of various second messenger enzymes, such as protein kinase C, using [^{3}H]phorbol dibutyrate (PDBu), activated adenylyl cyclase, using [^{3}H]forskolin and guanine nucleotide binding proteins, using [^{3}H]guanylyl-5'-imidodiphosphate (GppNHp). *In situ* hybridization experiments were also carried out using anti-sense and sense oligonucleotide probes complementary to the mRNA coding for a α-subunit of the G-protein Go.

Binding of [^{3}H]GppNHp was highest in dentate gyrus and the CA2-CA3 region, with no significant difference between controls and Alzheimer's patients. Binding of [^{3}H]forskolin was lower in the cortex ($P < 0.05$, Mann-Whitney U test) in Alzheimer's patients (37.9 ± 10.9 pmol/g) compared to controls (57.3 ± 5.9 pmol/g). The binding of [^{3}H]PDBu was lower ($P < 0.01$, Mann-Whitney U test) in the CA4 region of the hippocampus in Alzheimer's patients (463 ± 68 pmol/g) compared to controls (809 ± 91 pmol/g). Also, *in situ* hybridization studies revealed that the levels of expression of the mRNA coding for the α-subunit of the G-protein Go were not significantly different in any regions of the medial temporal lobe when Alzheimer's patients were compared to controls.

It has been shown, then, that levels of binding of [^{3}H]forskolin and [^{3}H]PDBu are lower in the medial temporal lobe, confirming the work of other groups working on other brain regions (Cole *et al*, 1988; Dewar *et al*, 1990), although in this case in a region of particular neuropathological interest. The levels of binding of [^{3}H]GppNHp (to G-proteins) have been found to be unchanged in this disease, a finding confirmed by the *in situ* hybridization study described here.

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36P SENSITIVITY TO LITHIUM IONS OF AGONIST-INDUCED INOSITOL PHOSPHATE ACCUMULATION IN HeLa CELLS

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Lithium has been widely employed in assays of agonist-induced phosphoinositide hydrolysis, since lithium ions inhibit the enzyme inositol monophosphatase and thereby cause the accumulation of [^{3}H]-inositol monophosphate ([^{3}H]-IP_1) in [^{3}H]-inositol prelabelled tissues (Berridge *et al.*, 1982). In mammalian brain 5-10 mM Li^+ is usually sufficient for a complete block of [^{3}H]-IP_1 breakdown under the assay conditions normally employed. In HeLa cells stimulated with histamine in the presence of 10 mM Li^+ [^{3}H]-IP_1 at first increases rapidly, but after 10-20 min reaches a plateau level (Tilly *et al.*, 1990a, b; Bristow & Young, 1991). We have investigated the reason for the failure of agonist-induced [^{3}H]-IP_1 to continue to accumulate with time in these cells.

HeLa cells were grown in DMEM supplemented with 10% bovine calf serum, penicillin and streptomycin. For the last 20-24 h of growth the cells were labelled with [^{3}H]-inositol in inositol-free DMEM containing 10 μM *myo*-inositol and 10% dialysed calf serum. Cells were transferred to Krebs-Henseleit solution before stimulation with agonist. Incubations were terminated with 10% perchloric acid and [^{3}H]-inositol phosphates separated by anion-exchange chromatography.

In the presence of 10 mM Li^+ [^{3}H]-IP_1 accumulation in response to both 1 μM and 1 mM histamine reached a plateau, although the level of the plateau was greater with 1 mM histamine. Levels of [^{3}H]-IP_2 and [^{3}H]-IP_3 were small after incubation with histamine for 15 min or longer. NaF (10 mM) added at the plateau phase of the response to 1 mM histamine induced a further increase in the level of [^{3}H]-IP_1. The level in the presence of histamine + NaF was 1.4 ± 0.2 fold (3) of that of the sum of the levels reached with histamine and NaF acting alone. Mepyramine (1 μM) added at the plateau phase of [^{3}H]-IP_1 accumulation in response to 1 mM histamine caused a decline in the level of [^{3}H]-IP_1, indicating that with 10 mM Li^+ breakdown of [^{3}H]-IP_1 is only partially inhibited. Preincubation with 10 mM Li^+ for more than 15 min before incubation with 100 μM histamine for 15 min did not increase the level of [^{3}H]-IP_1. A concentration effect curve to Li^+ showed that even with 30 mM Li^+ accumulation of [^{3}H]-IP_1 was not maximal. Carbachol-induced [^{3}H]-IP_1 accumulation was also greater with 30 mM than with 10 mM Li^+. The monophosphatase enzyme(s) in HeLa cells are thus less sensitive to Li^+ than the enzyme in mammalian brain. A similar lower sensitivity has been reported in other transformed cell lines (Raymond *et al.*, 1991, and references therein). In such cells in the presence of low concentrations of Li^+ the steady-state level of [^{3}H]-IP_1 will be approximately proportional to the rate of formation of [^{3}H]-IP_1 at that time.

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37P AN INVESTIGATION OF THE COUPLING OF SIGMA BINDING SITES TO A GUANINE NUCLEOTIDE BINDING (G) PROTEIN

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Multiple sigma sites have been postulated to exist in guinea-pig brains Rothman et al. (1990), whilst Itzhak (1989) and Beart et al. (1989) have suggested that sites identified by (+)-[^{3}H] 3-PPP exist in high and low affinity states and are linked to G proteins. The aim of the present investigation was to extend these studies and to examine the effect of the GTP analogue Gpp(NH)p on the displacement of ^{3}H-ditolyl guanidine (DTG) produced by sigma ligands.

Assays were performed using guinea-pig cerebral membranes in 50 mM Tris HCl buffer, the concentration of [^{3}H]-DTG was 3.4 nM and non-specific binding was displayed using 10 μM haloperidol. In some assays, 100 μM Gpp(NH)p was present. Results are means ± se mean ($n \geq 6$). IC_{50} values were obtained using GraphPAD InPlot and calculated for high and low affinity states when the Hill coefficient was less than unity.

	-Gpp(NH)p -log IC_{50} (M)			+Gpp(NH)p -log IC_{50} (M)		
	high	low	nH	high	low	nH
(+)-3 PPP	9.3±0.3	7.4±0.1	0.56±0.06		6.5±0.1	0.94±0.15
(+)-SK&F 10047	7.1±0.5	6.0±0.8	0.75±0.15		6.2±0.1	1.1±0.26
Haloperidol	10.5±0.4	7.8±0.2	0.36±0.10	10.3±0.5	7.8±0.2	0.54±0.10
Dextromethorphan	8.0±0.1	6.4±0.1	0.58±0.03		<5	-
DTG	9.1±0.2	7.3±0.2	0.50±0.07	8.3±0.1	6.3±0.3	0.68±0.06

In the absence of Gpp(NH)p, all compounds completely displaced specifically bound [^{3}H]-DTG with low Hill coefficients (nH) Addition of Gpp(NH)p reduced [^{3}H] DTG binding by 37.1%. The presence of Gpp(NH)p abolished the high affinity binding component for (+)-3-PPP, (+)-SK&F 10047 and dextromethorphan. In particular, the ability of dextromethorphan to displace [^{3}H]-DTG was dramatically reduced. These data indicate that the sigma sites are coupled to a G protein. In the presence of Gpp(NH)p, composite IC_{50} values resembled those reported previously for postulated sigma $_2$ sites (Rothman 1990). The ability of Gpp(NH)p to reduce specifically bound DTG, but not to remove high affinity binding requires clarification. Whether the relative lack of effect of Gpp(NH)p on the ability of haloperidol to displace DTG is indicative of multiple sigma sites requires evaluation.

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38P THE HUMAN α_2-C10 ADRENOCEPTOR CAN INTERACT WITH BOTH G_i2 AND G_i3 WHEN EXPRESSED IN RAT 1 FIBROBLASTS

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The DNA encoding the human α2-C10 (α2A) adrenergic receptor linked to the mammalian expression vector pDOL was introduced into Rat 1 fibroblasts by calcium phosphate precipitation and clones resistant to geneticin selected (Milligan *et al.*, 1991). One of these clones (1C) displayed high (2.5-3.0 pmol/mg membrane protein) specific binding of [^{3}H] yohimbine and bound oxymetazoline with considerably higher affinity than prazosin.

Activation of this receptor with UK14304 produced stimulation of high affinity GTPase activity, inhibition of adenylyl cyclase and stimulation of phospholipase D catalysed hydrolysis of phosphatidylcholine (MacNulty *et al.*,1991). All of these responses were abolished by pretreatment of the cells with pertussis toxin. Using either the ability of antisera directed against the C-terminal decapeptides of the α subunits of a range of individual G-proteins to interfere with agonist stimulation of high affinity GTPase or an assay in which G-protein a subunits become substrates for cholera toxin catalysed ADP-ribosylation only following contact with agonist-occupied receptors we demonstrated that in these cells the α2C10 receptor is able to physically interact with and activate both G_i2 and G_i3.

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39P VASOPRESSIN-STIMULATED PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE AND PHOSPHATIDYLCHOLINE HYDROLYSIS IN A10 VASCULAR SMOOTH MUSCLE CELLS

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It is well established that many vasoconstrictors, such as vasopressin, activate the hydrolysis of PtdIns(4,5)P_2 to produce the two second messengers, Ins(1,4,5)P_3 and diacylglycerol (DAG). Ins(1,4,5)P_3 stimulates the release of calcium from intracellular stores, whilst DAG activates protein kinase C (PKC). In many tissues a prolonged elevation of DAG is observed and this is believed to be derived from the sustained hydrolysis of an alternative phopholipid, phosphatidylcholine (PC)(Billah and Anthes 1990). The mechanism whereby the hydrolysis of this lipid is stimulated remains unclear, thus we have sought to determine the mechanism(s) involved in vasopressin-stimulated PC hydrolysis in A10 vascular smooth muscle cells.

For pharmacological characterisation of vasopressin-stimulated PtdIns(4,5)P_2 hydrolysis, total inositol phosphate accumulation ([^{3}H]IP) was determined in cells with [^{3}H]inositol (2μCi/ml). Ins(1,4,5)P_3 mass formation was measured by the method of Palmer*etal* (1989). Phosphatidylcholine hydrolysis was measured as the accumulation of phosphatidylbutanol (PtdBuOH) in [^{3}H]palmitic acid prelabelled cells (5μCi/ml), incubated in the presence of 30mM butanol.

Vasopressin ([Arg^8]-VP) stimulated both [^{3}H]IP and [^{3}H]PtdBuOH accumulation in A10 VSMC with similar EC_{50} values (IP=3.37±0.53nM; PtdBuOH=1.84±0.64nM ,n=3). A number of vasopressin analogues also yielded similar EC_{50} values for both responses (analogue, IP vs PtdBuOH, nM (n=3) ; vasotocin= 2.49±0.21 vs 2.78±1.3 ; [lys^8]-vasopressin = 3.47±.43 vs 2.78±1.5 ; oxytocin=626±17 vs 668±16), and gave the same rank order of potency ([Arg^8]-vasopressin=vasotocin=[lys^8]-vasopressin >> oxytocin). The V_{1a} selective antagonist (des-Gly^9-[β-Mercapto-cyclopentamethylene-propionyl^1, O-Et-Tyr, Val^4,Arg^8]-vasopressin), yielded similar IC_{50} values for the inhibition of vasopressin stimulated [^{3}H]IP and [^{3}H]PtdBuOH formation(IP=37.2±12nM ; PtdBuOH=20.6±9nM, n=3), suggesting that both PLC and PLD were activated through a single receptor subtype. The kinetics of Ins(1,4,5)P_3 and [^{3}H]PtdBuOH formation were also examined. Whilst Ins(1,4,5)P_3 increased rapidly in response to vasopressin (100nM), peaking at approximately 10 seconds (6.68±2.0 fold, n=3), there was a 10-15 second lag in the onset of the [^{3}H]PtdBuOH response. [^{3}H]PtdBuOH accumulation was linear up to 120 seconds and then levelled off between 2-5minutes, indicating that stimulated PLD activity was transient. The PKC activating phorbol ester, PMA, also stimulated [^{3}H]PtdBuOH formation in a dose dependent manner giving an EC_{50} value of 10.96± 3.16nM (n=3). The PKC inhibitor Ro-31-8220 inhibited both PMA and vasopressin-stimulated PtdBuOH accumulation. However the IC_{50} values were different by approximately 10 fold (PMA=1.85±0.79μM ; vasopressin = 27.50±0.65μM n=3).

The results here suggest that in vasopressin-stimulated A10 vascular smooth muscle cells, phosphatidylcholine breakdown is activated through a sequential pathway, involving initial PtdIns(4,5)P_2 hydrolysis and the intermediate activation of PKC. In addition, the kinetics of PtdBuOH formation suggest PC hydrolysis is not sustained but is a transient phenomenon which may only be partly responsible for the sustained generation of DAG.

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40P THE ROLE OF CYCLIC AMP IN THE CONTROL OF HEPATOCYTE PROLIFERATION BY HEPATOCYTE GROWTH FACTOR

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Hepatocyte growth factor (HGF) is the most potent known mitogen for adult rat hepatocytes in primary culture and is thought to have an important role in human liver growth and repair. However, little is known about the mechanism of action of HGF on hepatocytes. We have shown that human recombinant HGF (Nakamura et al, 1989) has no significant effect on the stimulation of adenylate cyclase by forskolin (100μM) or glucagon (10^{-9} to 10^{-6}M) although HGF caused a concentration-dependent (EC_{50} = 20 ng/ml) inhibition of basal adenylate cyclase activity in hepatocyte plasma membranes (Bushfield et al, 1991). This inhibition of basal activity was unusual in that there was no requirement for GTP and, therefore, it was not mediated by activation of the inhibitory G-protein Gi.

Since the adenylate cyclase system has been implicated in hepatocyte growth control, we examined (a) the effects of HGF on intracellular cyclic AMP levels in rat hepatocytes and (b) the effects of cyclic AMP elevation on the stimulation of DNA synthesis by HGF. Experiments were performed using either freshly isolated rat hepatocytes or hepatocytes which had been maintained in culture for 24 to 48 hours. HGF-induced DNA synthesis was monitored as incorporation of [^{3}H]-thymidine and the effects of HGF on cyclic AMP levels were measured as described (Whetton et al, 1983).

Treatment of hepatocytes with HGF (10 ng/ml) had no effect on the basal level of cyclic AMP (2.1 ± 0.04 pmol / 10^6 cells, mean ± SEM, n = 3) or on the increases induced by glucagon (100 nM), forskolin (10 μM) or IBMX (100 μM). Agents which elevated hepatocyte cyclic AMP levels caused an inhibition of HGF-stimulated DNA synthesis in primary hepatocyte cultures. Glucagon (100 nM), forskolin (1 μM), IBMX (100 μM), 8-bromo-cyclic AMP (300 μM) and dibutyryl-cyclic AMP (300 μM) completely inhibited HGF (10 ng/ml) -stimulated DNA synthesis. These results indicate that the stimulation of DNA synthesis in rat hepatocytes by HGF is not mediated by changes in intracellular cyclic AMP levels. However, agonist-induced elevation of hepatocyte cyclic AMP content results in inhibition of HGF responsiveness.

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41P PUTATIVE BRADYKININ BK_3 RECEPTORS IN THE GUINEA-PIG TAENIA CAECI AND TRACHEA

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In the guinea-pig airways, a putative novel BK_3 bradykinin receptor has been described which is insensitive to BK_1-antagonists and BK_2-antagonists of the [D-Phe^7]-BK series (Farmer *et al.*, 1989). Our preliminary studies showed that the bradykinin receptors of the guinea-pig taenia caeci are also insensitive to BK_1-antagonists and certain of these BK_2-antagonists (Field *et al.*, 1988). We therefore carried out experiments to investigate possible similarities in receptor types between the taenia caeci and the trachea. Both the epithelium-denuded trachea and the taenia caeci preparations were mounted isometrically in Krebs' solution containing mepyramine, guanethidine, cimetidine, atropine (1 μM) and hexamethonium (10 μM) at 37°C, and contractile responses were determined using serial doses of bradykinin administered in a random order. Estimates of apparent pK_B (App.pK_B) values in Table 1 were calculated for at least two concentration-levels of antagonist, from the Gaddum-Schild equation, and are the mean ± s.e.mean for 9 - 14 estimates.

Table 1 **Antagonist**	**Guinea-pig taenia caeci** App.pK_B (±s.e.mean)	**Guinea-pig trachea** App.pK_B (±s.e.mean)	P
[des-Arg^9, Leu^8]-BK	< 5.0	< 5.0	
D-Arg-[Hyp^3,D-Phe^7]-BK	5.89 (0.32)	5.94 (0.31)	>0.05
D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK	5.81 (0.35)	5.87 (0.22)	>0.05
D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK	8.42 (0.15)	8.94 (0.16)	<0.05

The pK_B estimates in Table 1. suggest that the receptors in the two preparations are very similar, but distinct from B_1-receptors and also from bradykinin-receptors in common BK_2 preparations where antagonists based on [D-Phe^7]-BK show higher affinity (pK_B *ca.* 7.5)(eg Field *et al.*, 1988). Thus, D-Arg-[Hyp^3,D-Phe^7]-BK and D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-BK were relatively inactive in both the taenia caeci and trachea, but responses in both preparations were readily antagonized by D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK, though with a lower affinity than that reported in some BK_2 preparations (Lembeck *et al.*, 1991; Perkins *et al.*, 1991). Whether the properties of the receptors in the trachea and taenia justify the creation of a distinct receptor class should, however, perhaps depend on evidence from other studies, such as of coupling mechanisms or structure.

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42P EFFECTS OF BRADYKININ ANTAGONISTS ON ANTIGEN-INDUCED PULMONARY DISTRESS, AIRWAY HYPER-RESPONSIVENESS AND EOSINOPHILIA IN GUINEA-PIGS CHRONICALLY CHALLENGED WITH OVALBUMIN

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A bradykinin (BK) B_2 receptor antagonist, DArg-[Hyp^3,DPhe^7]-BK (NPC 567), prevents antigen-induced airway hyperresponsiveness in allergic sheep (Solér *et al.*, 1990; Abraham *et al.*, 1991). Sensitized guinea-pigs, repeatedly exposed to antigen, also exhibit hyperresponsiveness to acetylcholine (ACh), and airway infiltration by eosinophils (Ishida *et al.*, 1990). We have examined the effects of NPC 567 and DArg-[Hyp^3,Thi^5,DTic^7,Tic^8]-BK (NPC 16731), a more potent BK antagonist (Farmer *et al.*, 1991; Kyle *et al.*, 1991), on these phenomena.

Female Hartley guinea-pigs (200-250 g) were sensitized to ovalbumin (OA, 10 µg + 10 mg $Al(OH)_3$, i.p.). Starting 14 days later, animals inhaled aerosolized OA (0.5 mg/ml), under cover of diphenhydramine (8 mg), twice weekly for 4-5 weeks. Aerosolized NPC 567 (30 mg/ml) or NPC 16731 (1.5 mg/ml) were administered by inhalation for 30 min prior to each antigen challenge. NPC 567 (6 mg/ml) or NPC 16731 (0.3 mg/ml) were also co-administered with antigen. 24-48 Hours after the final challenge, airway responsiveness to ACh (i.v.) was determined by measuring changes in pulmonary inflation pressure (PIP) in animals anaesthetized with urethane. Airway histological sections were prepared from representative animals, stained with haematoxylin and eosin, and airway (not blood vessel) eosinophils in 10 random fields counted.

OA challenge caused marked respiratory distress. At week 5, NPC 16731, but not NPC 567, significantly increased time to onset of dyspnea from 6.2 ± 1.5 min in controls to 12.0 ± 1.7 min. Guinea-pigs treated with BK antagonists rarely exhibited cyanosis, and recovered more quickly than controls after challenge. Chronic challenges with OA caused airway hyperresponsiveness to ACh, producing a 50% increase in the maximum PIP response to ACh (Table 1). The BK antagonists both significantly inhibited the development of airway hyperresponsiveness (Table 1).

Treatment group	Control (6)	Antigen (OA) (7)	NPC567 (6)	NPC567 + OA (5)	NPC16731 (6)	NPC16731 + OA (6)
Max. ↑ in PIP (cmH_2O)	33.0 ± 3.8	49.6 ± 3.5	38.6 ± 2.9	41.2 ± 2.9	37.0 ± 5.5	41.2 ± 1.7

Airway eosinophilia occurred in OA-challenged animals. (e.g., control lobar bronchi had 2.6 ± 1 eosinophils/field, compared to 20.3 ± 3.1 after OA). Both BK antagonists significantly reduced the numbers of eosinophils in trachea, right main bronchus and lobar bronchi, and parenchyma.

These data suggest that BK is causally involved in acute antigen-induced pulmonary distress and chronic airway hyperresponsiveness. The ability of BK antagonists to reduce airway hyperresponsiveness and eosinophilia suggests that these phenomena may be related.

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43P ANGIOTENSIN II-INDUCED CONTRACTIONS OF THE ISOLATED PREPARATION OF RAT UTERUS ARE MEDIATED THROUGH THE AT_1 RECEPTOR SUBTYPE

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In rat uterus, angiotensin II (AII) receptors exist in two subtypes (Whitebread et al, 1989), which are now referred to as AT_1 and AT_2 (Bumpus et al, 1991). It has been reported that AII contracts uterine smooth muscle by two different mechanisms, one of which is independent of the electrical state of the membrane (Freer & Smith, 1976). The aim of this study was to characterise the AII-induced contractions of standard and potassium-depolarised rat uterus, using the AT_1 and AT_2 selective antagonists, DuP 753 and EXP655 respectively (Chiu et al, 1989) and the disulphide reducing agent, dithiothreitol (DTT). DTT has been shown to inhibit radioligand binding to AT_1 sites (Whitebread et al., 1989) and to abolish AT_1 receptor-mediated contractions in rabbit aorta (Fleisch et al, 1973).

The studies used female Lister-hooded rats (200-250g), which were pretreated with stilboestrol (0.1mg/kg ip). The uteri were isolated and mounted in de Jalons solution, aerated with 5%CO_2 / 95%O_2 at 28^{0}C. Contractions to AII were recorded isometrically. In the standard preparation, to avoid tachyphylaxis, single concentrations of AII were applied serially at intervals of 20 min. In the depolarised preparation, tissues were initially contracted with a depolarising solution containing 151mM K^+, 0.18mM $CaCl_2$. Under these conditions, tachyphylaxis does not occur and AII was applied cumulatively. On each tissue, AII curves were established prior to and following 30 min pre-incubation with single concentrations of DuP 753, EXP655 or DTT. Indomethacin (30μM) was present throughout. Where appropriate, antagonist potency was expressed as estimates of mean pK_B (with 95% confidence limits), obtained from Schild analysis.

In the standard preparation, DuP 753 (0.01 - 0.3μM) competitively antagonised the AII-induced contractions. The mean pK_B was 8.75 (8.64-8.87); Schild slope = 0.92±0.10 (not significantly different from 1). DuP 753 also antagonised the AII-induced contractions of the depolarised preparation. The mean pK_B was 8.61 (8.45-8.78); Schild slope = 1.16±0.19 (not significantly different from 1). In contrast, 3μM EXP655 failed to antagonise the AII-induced contractions of either the standard or depolarised rat uterus preparations. These results indicate that the AII-induced contractions of rat uterus are mediated through the AT_1 receptor subtype.

Preincubation with DTT (1mM) failed to modify the AII-induced contractions of either the standard or depolarised rat uterus preparation. The lack of effect of DTT to inhibit the AT_1 receptor-mediated contractions in this tissue contrasts with the ability of DTT to inhibit to AT_1 receptor-mediated contractions in rabbit aorta. This discrepancy remains to be explained.

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44P ANALYSIS OF MUSCARINIC RECEPTORS IN GUINEA-PIG OESTROGEN-DOMINATED UTERUS, *IN VITRO*

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In contrast to most smooth muscles, contraction of guinea-pig oestrogen-dominated uterus may be mediated by M_2 receptors (Eglen et al., 1989). Dorje et al., (1990), alternatively, have suggested that the muscarinic receptor in the uterus resembles the m4 receptor, rather than the M_2 subtype. To clarify these contrasting reports, the muscarinic receptor present in this tissue has been further characterized using both ligand binding and molecular biological approaches.

In radioligand binding studies, the muscarinic receptor present in the guinea-pig oestrus uterus was compared to the putative m4 muscarinic receptor present in rabbit lung (Lazareno et al., 1990). Binding of [^{3}H]-N-methylscopolamine to membrane fractions were studied ([^{3}H]-NMS concentration = 0.2 nM) as described in Lazareno et al., (1990). In molecular biological studies, mRNA from uterus was probed with radiolabelled genomic DNA that encoded either the entire human muscarinic receptor sequence (m1, m2, m3 and m4) or the region encoding the third intracellular loop. Subsequent Northern blot analysis was then performed under high stringency conditions.

In competition binding assays (performed in parallel), gallamine, methoctramine, pirenzepine and himbacine displayed -log IC_{50} values of 7.0, 8.5, 6.3 and 7.4 respectively (n=3; s.e. mean < 0.1) at uterine membranes in comparison to -log IC_{50} values of 6.0, 7.2, 6.8 and 7.2 respectively at m4 sites in rabbit lung membranes. All Hill coefficients were not significantly ($P<0.05$) different from unity. [^{3}H]NMS possessed a K_d value of 54.3 ± 10 and 26.2 ± 11 pM (mean ± s.e. mean, n=3) in guinea-pig uterus and rabbit lung respectively. The rate of dissociation of [^{3}H]NMS from the m4 receptor ($t_{½}$ = 31.5 min) was slower than that from the uterus M_2 receptor ($t_{½}$ = 6 min). The oestrogen-dominated uterus exhibited no message for m1 or m3 mRNA, but two messages for m2 mRNA species (6.8 and 3.6 kilobases) were detected by Northern blot analysis. Additionally, a 6.5 kb message for the m4 mRNA was also observed.

The radioligand binding profile of the guinea-pig uterus was similar to that previously reported (Eglen et al., 1989) at cardiac M_2 receptors, but dissimilar to that seen at the rabbit lung m4 receptor. The molecular biological studies suggested that m2 and m4 mRNA species were expressed with an excess of m2 over m4 mRNA. Taken together, these data may suggest that the guinea-pig oestrogen-dominated uterus expresses predominantly M_2 muscarinic receptors, which appear to mediate contraction.

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45P COMPARISONS OF THE SELECTIVE CCK AGONISTS A71378 AND A72962 IN GUINEA-PIG ILEUM

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Results of functional comparisons of gastrin/CCK receptor agonists and antagonists in guinea-pig ileal longitudinal muscle myenteric plexus (GPI) are consistent with the view that responses are mediated by CCK_A and CCK_B receptors (Lucaites et al., 1990; Patel and Spraggs, 1991). Recent studies in GPI have suggested that the predominant response is a sustained contraction mediated by CCK_A receptors. In addition, using devazepide (100nM) to selectively antagonise CCK_A mediated contractions, it is possible using CCK-4, to unmask a transient contractile response which appears to be mediated by CCK_B receptors (Patel and Spraggs, 1991). The availability of the CCK_A and CCK_B receptor selective peptide agonists, A71378 and A72962, (Lin et al., 1990) has provided an opportunity to further characterise gastrin/CCK receptors in GPI. Guinea-pig ileal longitudinal muscle strips were prepared and used as previously described (Patel and Spraggs, 1991). Table 1 summarises the effects of gastrin/CCK peptides in GPI (with and without devazepide 100nM). Both A71378 and A72962 produced a contractile response similar to the sustained phase of contraction produced by CCK-8, the transient phase being absent. The mechanisms of these responses in GPI were investigated further using tetrodotoxin (TTX) and atropine. TTX (300nM) abolished the responses to all 4 peptides tested. Atropine (1μM) abolished the responses to CCK-4, whilst at higher concentrations, (10μM) it produced only 50± 15%, 60± 12% and 70±8% inhibition of CCK-8, A71378 and A72962 induced contractions respectively (n=4 each) suggesting that acetylcholine was the sole mediator of CCK-4 responses but an additional transmitter was also involved in the responses to CCK-8, A71378 and A72962.

Table 1 The effect of gastrin/CCK peptides on GPI

Agonist		CCK-8	CCK-4	A71378	A72962
Control	EC_{50} (nM)	2.0 (0.9 - 4.1)	176.9 (32.6 - 961.0)	0.7 (0.5 - 0.9)	129.0 (80.7 - 206.9)
	Emax (g)	0.98 ± 0.12	0.56 ± 0.06†	0.81 ± 0.06	0.92 ± 0.10
Devazepide (100nM)	EC_{50} (nM)	1508.9 (985.1 - 2311.2)	78.8 (47.3 - 131.5)	253.0 (80.3 - 797.1)	3.9 (2.2-6.9)*
	Emax (g)	0.60 ± 0.06	0.53 ± 0.11	0.64 ± 0.17	0.23 ± 0.03

Values shown are the mean values with 95% confidence interval (EC_{50}) and S.E.M. (Emax) (n=4-6). * Denotes EC_{50} value for A72962 expressed as 50% of its own maximum. Although Emax for A72962 was reduced, threshold concentration remained the same. † Maximum response at highest concentration employed.

In conclusion A71378 appears to be a CCK_A receptor selective agonist. A72962 produced sustained contractions of GPI, with a mechanism of action similar to that of CCK-8 and A71378. In the presence of L-364,718, EC_{50} values for CCK-4 and A72962 were reduced and A72962 became a partial agonist. Thus, whilst A72962 appeared potent at CCK_B receptors, it had low efficacy in GPI and therefore its effects appear to be mediated predominantly via CCK_A receptors. These studies suggest that A72962 is not a selective CCK_B receptor agonist.

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46P 5-HT STIMULATES ADENYLYL CYCLASE IN RAT OESOPHAGEAL TUNICA MUSCULARIS MUCOSAE (TMM) VIA NON-CLASSICAL 5-HT RECEPTORS

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Relaxation of rat oesophageal TMM has been described recently as a screen for agents acting at non-classical 5-HT receptors ($5\text{-}HT_4$ receptors; Baxter *et al.*, 1991). The TMM offers advantages over other assay systems (guinea pig ileum, Craig and Clarke, 1990; guinea pig hippocampus, Shenker *et al.*, 1987) due to the postjunctional location of the receptor and the absence of additional 5-HT receptor subtypes. Hitherto, non-classical 5-HT receptors have been demonstrated to be coupled positively to adenylyl cyclase in the CNS (guinea pig hippocampus and mouse colliculus, Dumuis *et al.*, 1988) and in the atrial appendage of man (Kaumann *et al.*, 1990). The coupling nature of this receptor in smooth muscle tissue, which we now describe, has not been reported previously.

Rat (male, Sprague-Dawley, 300-450 g) oesophageal TMM were cross-chopped (0.35mm), collagenase-treated (2.5 mg ml^{-1}, 20 min), and equilibrated in Tyrode's solution (37°C). Following incorporation of [^{3}H]-adenine (2 mCi ml^{-1}, for 40 min) , washed slices (100 µl) were pipetted into mini-vials containing Tyrode's solution, IBMX (1mM), methysergide (1 µM), cocaine (30 µM), corticosterone (30 µM) and antagonist or vehicle. After 30 min, 5-HT or renzapride was added (10 min stimulation, 300 µl final volume) and the mixture gassed with O_2/CO_2 (95/5 %) . The [^{3}H]-cyclic AMP formed was extracted and isolated using a single column method similar to that described by Alvarez and Daniels (1990), and was measured by liquid scintillation counting.

5-HT and renzapride stimulated cyclic AMP production, concentration-dependently, to a maximum of 260 % and 190 % of basal levels (cpm/vial, basals: mean ± s.e.mean, 1773 ± 53, 5-HT; 1123 ± 74, renzapride), with -log EC_{50} values of 7.09 ± 0.13 and 6.78 ± 0.2. Agonist-induced cyclic AMP formation was inhibited concentration-dependently by ICS 205-930 (tropisetron) and SDZ 205 557, with 100µM producing complete inhibition. Rightward displacement of concentration-effect curves for 5-HT in the presence of ICS 205 930 and ¶SDZ 205 557 (both at 3 µM) allowed estimation of pA_2 values for these antagonists of 6.7±0.3 and 7.7±0.2, respectively (n=3).

Compared with the values obtained for 5-HT- and renzapride- induced stimulation of adenylyl cyclase, and inhibition by ICS 205-930, in colliculus and hippocampus (Dumuis *et al.*, 1988; Shenker *et al.*, 1987), the present data correlate well. Compared with relaxant responses to 5-HT and renzapride in rat TMM, the -log EC_{50} values are lower, compatible with loss of post-cyclic AMP amplification (reduced effective receptor reserve). The affinity constant for ICS 205-930 agrees with values obtained for relaxant responses (pA_2 = 6.7; Baxter *et al.*, 1991). It is concluded, therefore, that non-classical 5-HT receptors, provisionally termed $5\text{-}HT_4$ receptors, located on smooth muscle cells of rat TMM, are, like their equivalent in rodent CNS and human heart, positively coupled to adenylyl cyclase.

¶SDZ 205 557: 2-diethylaminoethyl-(2-methoxy-4-amino-5-chloro)-benzoate hydrochloride.

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47P ACTIONS OF BENZIMIDAZOLONE DERIVATIVES AT PUTATIVE 5-HT_4 RECEPTORS IN RAT OESOPHAGUS

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BIMU 1 (endo-N-(8-Methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1H-benzimidazole-1-carboxamide hydrochloride), BIMU 8 (endo-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1H-benzimidazole-1-carboxamide hydrochloride) and DAU 6215 (N-(endo-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-2-oxo-1H-benzimidazol-1-carboxamide, hydrochloride) are novel benzimidazolone derivatives which appear to possess agonist activity at 5-HT_4 receptors in the CNS and gastrointestinal tract (Dumuis *et al.*, 1990; Rizzi *et al.*, 1990). The present study examines BIMU 1, BIMU 8 and DAU 6215 in rat oesophageal tunica muscularis mucosae. This preparation possesses a homogeneous population of 5-HT_4 receptors which mediate a well defined relaxant response to 5-HT (Baxter *et al.*, 1991).

Rat oesophageal tunica muscularis mucosae were suspended under 2.5 mN tension in tissue baths containing oxygenated Tyrode's solution at 37°C as described by Baxter *et al.* (1991). Relaxant responses to the cumulative addition of agonists were exposed after contracting tissues submaximally with carbachol (3 μM).

BIMU 1, BIMU 8 and DAU 6215 evoked relaxation of the carbachol contracted rat oesophagus. Concentration-effect curves for DAU 6215 were monophasic, whereas those to BIMU 1 and BIMU 8 consisted of both high and low potency phases. Over the high potency phase, BIMU 1 and BIMU 8 behaved as potent but partial agonists (EC_{50}'s [95% CL] = 8.0 [7.8-8.1] and 7.9 [7.7-8.8]; intrinsic activities of 0.7 and 0.9 respectively) with respect to 5-HT (EC_{50} [95% CL] = 8.2 [8.1-8.3]) and were antagonized competitively by ICS 205930 (pA_2 estimates of 6.8 and 6.7). Desensitization of 5-HT_4 receptors with 5-methoxytryptamine (10 μM for 90 minutes) abolished the high potency phase of concentration-effect curves to BIMU 1 and BIMU 8, but full responsiveness to DAU 6215 remained. Further studies revealed that DAU 6215 antagonized carbachol (pA_2 [95% CL] = 6.21 [6.10-6.30]) demonstrating that cholinergic blockade mediates its relaxant activity. However, despite its anticholinergic action, DAU 6215 (1 μM and 3 μM) acted as a competitive antagonist toward 5-HT yielding pA_2 estimates [95% CL] of 6.7 [6.5-6.8] and 6.5 [6.2-6.8] respectively.

The present study shows that BIMU 1, BIMU 8 and DAU 6215 possess affinity for the 5-HT_4 receptor mediating relaxation of the rat oesophagus. BIMU 1 and BIMU 8 act as partial agonists relative to 5-HT, whereas DAU 6215 acts as a competitive antagonist. Although DAU 6215 and ICS 205930 are equally affine at the 5-HT_4 receptor, the use of DAU 6215 over ICS 205930 may be limited by its higher affinity for muscarinic cholinoceptors.

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48P CHARACTERIZATION OF MUSCARINIC RECEPTORS IN CANINE FEMORAL VEIN *IN VITRO*

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Muscarinic M_1 receptors have been characterized predominantly in neuronal tissue (see Hulme et al., 1990 for review) although it has been suggested that postjunctional M_1 receptors mediate contractions of canine saphenous vein (Eglen et al., 1990). In the present study, postjunctional muscarinic receptor subtypes in canine femoral vein has been characterized. This tissue has been shown (Rubanyi & Vanhoutte, 1988) to contract, but not relax, in response to muscarinic agonists.

Rings (2 mm) of canine (mongrel, male, 6-8 kg) femoral vein were suspended under 1.0 g preload in Krebs-Henseleit solution (pH 7.4, 37°C) for isometric recording. Concentration-response curves were performed in a cumulative fashion and antagonist equilibration periods of 60 min were used in studies to derive the pA_2 value. In separate experiments, receptor inactivation studies (Furchgott & Bürsztyn, 1967) were performed to determine the apparent agonist affinity (-log K_D) using 3 μM phenoxybenzamine (20 min exposure). All values are mean, s.e. mean <10%, from 4-8 animals.

The rank order of agonist potency (-log EC_{50}) was: acetylcholine (5.21) > (+)cis-dioxolane (4.80) OXA-22 (4.92) > carbachol (4.52) methacholine (4.32). Pilocarpine, bethanechol and McN-A-343 were inactive as agonists (1 nM-100 μM). The apparent affinities (-log K_D), determined from receptor inactivation by antagonist were: OXA-22 (5.04) > arecoline (4.66) ≥ acetylcholine (4.60) > carbachol (4.51) > pilocarpine (4.23) = McN-A-343 (4.23) > methacholine (3.25) > bethanechol (3.16). There was no change in the potency of carbachol in two consecutive curves, constructed at 60 min intervals (curve 1 -log EC_{50} = 4.21; curve 2 -log EC_{50} = 4.23) suggesting that the tissue did not display desensitisation under the conditions employed. The pA_2 values (mean ± SEM, n=4), with Schild slopes in parenthesis, for atropine, pirenzepine, methoctramine and (±)para-fluoro-hexahydro-siladifenidol were 9.09 ± 0.08 (0.92 ± 0.11), 8.26 ± 0.11 (1.07 ± 0.10), 6.05 ± 0.09 (1.01 ± 0.07) and 7.11 ± 0.11 (1.08 ± 0.07), respectively.

The similarity between agonist potency (-log EC_{50}) and apparent affinity (-log K_A), suggests an absence of a substantial receptor reserve associated with the contractile response. The pA_2 values indicate that stimulation of the M_1 muscarinic receptor subtype mediates contraction. In this respect, the canine femoral vein resembles the canine saphenous vein in terms of the muscarinic receptor population (Eglen et al., 1990) and provides a further example of postjunctional, smooth muscle M_1 muscarinic receptors.

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49P SOURCES OF CALCIUM MOBILISED BY α-ADRENOCEPTORS IN HUMAN SUBCUTANEOUS RESISTANCE ARTERIES

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The α_2-adrenoceptor has been located postjunctionally in human subcutaneous resistance arteries (Nielsen *et al* 1989). Little is known of the mechanism of action of the postjunctional α_2-adrenoceptor or the relative importance of either the extracellular or intracellular pool of calcium ions. To investigate which source of calcium was of more importance, *in vitro* studies were undertaken on human subcutaneous resistance arteries (internal diameter 126-412μm).

13 resistance arteries were obtained from 10 patients undergoing surgery and studied using a myograph (Mulvany & Halpern, 1977). Maximum responses to 118mM potassium depolarising solution (KDS), noradrenaline (NA) (10μM) and azepexole (BHT) (10μM) an α_2-adrenoceptor agonist, were ascertained in physiological saline solution (PSS) containing 2.5mM calcium, or calcium-free PSS containing 1mM BAPTA. In other experiments, the maximum response to caffeine (20mM), NA (10μM) and BHT (10μM) was determined in the absence and presence of ryanodine (10μM, incubated for 30 min).

Table 1.

	BHT (n=5)	NA (n=6)
PSS	41.4±6.0	103±5.9
Ca-free PSS	3.43±1.31	26.6±4.7
p	<0.05	<0.05

Table 2.

	NA (n=7)	BHT (n=6)	Caffeine (n=7)
PSS	92.9±8.6	17.8±5.3	32.3±5.5
+Ryanodine	102.4±16.6	14.7±5.7	8.16±1.6
p	ns	ns	<0.05

Results are expressed as a percentage of the KDS response ±s.e.m. (mean tension produced by KDS = 2.9±0.41N/m, n=13). Responses were compared using a Wilcoxon matched pairs test.

Table 1 shows the effect of removal of extracellular calcium on the responses to NA and BHT. Responses to NA were partially inhibited in contrast to the almost complete inhibition of responses to BHT. Table 2 shows the effect of ryanodine; the response to caffeine was markedly attenuated, but the effect of NA and BHT was not significantly altered.

It would appear that the contraction elicited by α_2-adrenoceptor activation predominantly arises from calcium influx. Contraction induced by NA, a non-selective α-adrenoceptor agonist, may involve mobilisation of internal calcium stores. However, ryanodine has no effect on the response to NA, although it profoundly inhibits the caffeine-induced contraction. This suggests that the intracellular store of calcium used by NA may differ from that mobilised by caffeine.

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50P EFFECTS OF RS-15385-197 AND OTHER α2-ADRENOCEPTOR ANTAGONISTS ON TAIL SKIN TEMPERATURE IN UNANAESTHETIZED RATS

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RS-15385-197 is a potent α_2-adrenoceptor antagonist *in vitro* and *in vivo* (Clark *et al.*, 1989; Brown *et al.*, 1990). During the course of our *in vivo* studies we noticed a palpable increase in the temperature of the tail in rats treated with this agent, and therefore decided to examine this phenomenon in more detail.

Male Sprague-Dawley rats weighing 200-320g were used. Experiments were conducted at an ambient temperature (T_A) of 18.5-20.0 °C, in the room in which they were housed. Rats were lightly restrained in well-ventilated perspex cages (length 18 cm, width 12 cm, height 10 cm). Core temperature (T_C) was measured with a rectal thermocouple, and tail skin temperature (T_S) was measured with a flat disc thermocouple (diameter 7 mm) taped to the dorsal surface of the tail 7 cm from the tip. Readings of T_A, T_C and T_S were taken at 5 min intervals, beginning 1h after preparing the animals. Immediately after the fourth reading the rats were injected subcutaneously with drug or vehicle (n = 6 per group), and the responses were followed over 1h.

In the group of rats studied (n = 126), T_S was 21.0 ± 0.2 °C and T_C was 38.2 ± 0.0 °C. RS-15385-197 (1 mg/kg, s.c., n = 6) produced a rapid elevation in tail skin temperature to a peak of 29.1 ± 0.7 °C ($P < 0.001$), 10 min after injection. T_C fell slightly, by 1.0 ± 0.1°C. The T_S response was dose-related over the dose range tested (0.01-1 mg/kg, s.c.), with an ED_{50} of 17 μg/kg, s.c. (n = 6 per dose). Other α_2-adrenoceptor antagonists were also effective to differing extents. The maximum increases in T_S in response to a dose of 1 mg/kg, s.c., were as follows (n = 6 per drug): RS-15385-197 (+9.6 ± 0.8 °C) > yohimbine (+9.0 ± 1.0 °C) > WY-26703 (+7.9 ± 1.3 °C) > piperoxan (+5.6 ± 1.7 °C) > idazoxan (+4.6 ± 1.1 °C) > imiloxan (+4.1 ± 1.3 °C) > SKF 104078 (+2.0 ± 1.9 °C) > BDF-6143 (+ 1.3 ± 0.8 °C). Prazosin (0.3 mg/kg, s.c.) did not increase T_S; propranolol (10 mg/kg, s.c.) evoked a small increase in Ts (+2.9 ± 1.0 °C). Pentolinium (2-10 mg/kg, s.c.) elicited a dose-related increase in T_S, which was elevated by 4.4 ± 1.3 °C after a dose of 10 mg/kg. T_C was reduced in a dose -related manner; a dose of 10 mg/kg reduced T_C by 3.8 ± 0.8°C. This effect, together with a lowering of blood pressure, could explain why ganglionic blockade failed to elevate T_S to the same extent as RS-15385-197. Drug vehicles (1 ml/kg, s.c.) had no effects on T_S or T_C.

These data indicate that α_2-adrenoceptor antagonists elevate tail skin temperature in the rat. This phenomenon has previously been noted for yohimbine (Wilson & Fregly, 1985). It is not known whether this is a central or peripheral effect, or whether this is due to an increase in total tail blood flow, redistribution of blood flow from deep vessels (either arterial or venous) to the tail surface, or opening of arteriovenous anastomoses. In view of the positive results with pentolinium, and the negative results with prazosin, our working hypothesis is that vasoconstriction within the tail, which prevents heat loss at an ambient temperature of around 19 °C, involves post-junctional vascular α_2-adrenoceptors. This suggestion is supported by indirect evidence from *in vitro* studies of this vascular bed (Templeton *et al.*, 1989).

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51P DRUG-INDUCED ENHANCEMENT OF CONTRACTIONS TO CLONIDINE IN RAT TAIL ARTERY RINGS *IN VITRO*

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Alpha$_2$-adrenoceptors occur on smooth muscle in the rat tail artery (MacLean & McGrath, 1990; Rajanayagam et al., 1990). These receptors are elusive and various techniques have been used to reveal them. This study investigated some of the conditions necessary to demonstrate enhanced postsynaptic alpha$_2$-adrenoceptor mediated responses to clonidine (CLON). Segments (3-4 mm) of proximal tail artery from male Wistar rats (150-200 g) were suspended between pairs of stainless steel hooks, inserted through the lumen, in Krebs buffer (37°C), gassed with 95% O_2/5% CO_2. The resting tension on each artery ring was set at 1 g. After 2 h equilibration, responses to drugs were recorded isometrically. Responses to a standard submaximal concentration (10 nM) of CLON were examined in the absence and presence of submaximal concentrations of phenylephrine (PE, 0.5 μM), potassium chloride (KCl, 0.03 M) or vasopressin (VP, 0.4 miu ml^{-1}). The standard response to CLON was also examined in rings precontracted with PE (0.5 μM), KCl (0.03 M) or VP (0.4 miu ml^{-1}) and then relaxed with isobutylmethylxanthine (IBMX, 10 μm). The response to CLON (10 nM) was enhanced in the presence of PE (0.5 μM) but not in the presence of KCl (0.03 M) or VP (0.4 miu ml^{-1}). When the artery ring was precontracted with PE, KCl or VP and relaxed with IBMX (10 μM), the superimposed response to CLON (10 nM) was enhanced respectively by 115 ± 17% (mean ± s.e. mean, 0.05>P>0.01, n=7), 933 ± 15%, (P<0.001, n=6) and 121 ± 15% (0.05>P>0.01, n=7). In each case the enhanced response to CLON was similar to that produced by the precontracting agonist alone (PE/CLON, R=0.97, P<0.001; KCl/CLON, R=0.93, P<0.001: VP/CLON, R=0.94, P<0.001). This enchanced response to CLON was antagonised by yohimbine (50 nM) but not by prazosin (2 nM). The enhanced response to CLON, in the presence of precontracting agonists may have occurred because CLON inhibited an inhibitory, cyclic nucleotide-mediated mechanism that was amplified by IBMX-induced blockade of phosphodiesterase.

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52P THE EFFECT OF A CALCITONIN GENE-RELATED PEPTIDE ANTAGONIST (CGRP$_{8-37}$) AND INDOMETHACIN ON BLOOD FLOW INDUCED BY INTRADERMAL CAPSAICIN

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Capsaicin stimulates a dose-dependent local increase in microvascular blood flow when injected intradermally (id) in rabbit skin (Buckley et al., 1990) which is partially, but significantly, inhibited by an anti-human CGRP antibody (Buckley et al., 1991). We have now investigated the ability of the CGRP receptor antagonist CGRP$_{8-37}$ (Chiba et al., 1989) and the prostaglandin synthesis inhibitor indomethacin to influence capsaicin-induced blood flow in rabbit skin.

Local blood flow changes induced by test agents (0.1ml/site id) were measured over 15 min in the shaved dorsal skin of anaesthetised (pentobarbitone sodium 30 mg/kg) New Zealand White rabbits (male, 2.5-3 kg) by multiple site ^{133}Xenon clearance (Brain et al, 1985). The selective antagonist activity of CGRP$_{8-37}$ was tested. CGRP$_{8-37}$ (3x10^{-10}mol/site) inhibited vasodilator responses to human αCGRP and βCGRP, but not to vasoactive intestinal peptide (VIP). Results expressed as % increase in blood flow compared with saline-injected sites, mean ± s.e. mean n=7-8, are:- human αCGRP (3x10^{-12}mol) 43±11%, human αCGRP + CGRP$_{8-37}$ -2±5%; human βCGRP (3x10^{-12}mol) 48±8%, human βCGRP + CGRP$_{8-37}$ 18±9%; VIP (10^{-11}mol) 58±10%, VIP + CGRP$_{8-37}$ 49±5%.

The effect of CGRP$_{8-37}$ on capsaicin (Cap)-induced blood flow is shown in Table 1. Indomethacin has no effect on the increased blood flow induced by CGRP in rabbit skin (Brain et al., 1985) and Table 1 shows that indomethacin (Indo), at doses which inhibit arachidonic acid (AA)-induced blood flow, has no significant effect on capsaicin-induced blood flow.

Table 1. Effect of CGRP$_{8-37}$ and indomethacin on skin blood flow (% change compared with saline-injected sites).

Agent (mol/site)	mean s.e.mean, n=6	Bonferroni's modified t-test (compared with vasodilator alone)
Cap (10^{-7})	49.0±5.6	-
Cap (10^{-7}) + CGRP$_{8-37}$ (3x10^{-10})	31.9±6.4	p<0.05
CGRP$_{8-37}$ (3x10^{-10})	0.4±3.3	-
Cap (10^{-7}) + Indo (3x10^{-9})	42.3±5.7	not significant
AA (3x10^{-9})	44.7±8.0	-
AA (3x10^{-9}) + Indo (3x10^{-9})	14.5±4.5	p<0.01
Indo (3x10^{-9})	-2.1±3.4	-

CGRP$_{8-37}$ significantly inhibits capsaicin-induced blood flow. The results suggest that capsaicin stimulates the release of vasodilator quantities of CGRP. This is in agreement with our earlier studies using an anti-CGRP antibody (Buckley et al., 1991). Further, we suggest that prostaglandins do not play a key role in capsaicin-induced vasodilatation in rabbit skin.

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53P NEUROPEPTIDE Y RECEPTOR SUBTYPES: POSSIBLE LOCATION AND FUNCTION IN THE RAT MESENTERIC ARTERIAL BED

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The existence of pre- and postsynaptic Neuropeptide Y (NPY) receptors involved in the inhibition of periarterial nerve stimulated (PNS) noradrenaline (NA) release and potentiation of the concomitant increase in perfusion pressure in the mesenteric artery have been demonstrated (Westfall *et al.* 1990). These receptors were designated Y2 and Y1 respectively, based on the ability of C-terminal fragments of NPY to mimic the former but not the latter. This study further investigates the involvement of these putative receptor subtypes in the actions of NPY in this system.

The mesenteric arterial bed from male Sprague-Dawley rats (300-375g) was isolated and removed. The superior mesenteric artery was cannulated, placed in an organ bath maintained at 37^{0}C and perfused at a rate of 5ml min^{-1} and superfused at a rate of 0.5 ml min^{-1} with an aerated Krebs-Bicarbonate buffer containing desipramine (1μm) and corticosterone (80μm) according to the method previously described (Westfall *et al.* 1990).

[Leu^{31}Pro^{34}]NPY (LP.NPY), an analogue with reputed specificity at Y1 receptors (Fuhlendorff *et al.* 1990) reduced PNS (16Hz, 60V, 2msec duration, 30sec) release of NA (Fractional releasing 5min^{-1} ± s.e. mean: control, 3.4± 0.3; 10^{-9}M, 2.4± 0.2; n=9 P<.05), suggesting the presence of a presynaptic Y1 receptor. A reduction in the concomitant PNS increase in perfusion pressure was demonstrated (ΔPerfusion pressure, mmHg ± s.e. mean: control 77.7±7.0; 10^{-9}M,46.2±5.4). LP.NPY was observed to potentiate the increase in perfusion pressure elicited by exogenously administered NA supporting the concept of a Y1 postsynaptic receptor (Table 1a). [11-36]NPY also potentiated (latency of onset 8min; maximum increase 30min) the increase in perfusion pressure produced by NA indicating that the Y2 subtype may also participate in the enhancement of the contractile response (Table 1b). The potentiating actions of LP.NPY and [11-36]NPY were modified by Phentolamine (Phen), Benextramine (Benxt), and the supposed novel NPY antagonist (Tatemoto, 1990) PYX1 (Table 1a&b).

Table 1a: NA response ± LP.NPY(10^{-10}M)

Pretreatment	ΔPerf. Press. (mmHg) ± s.e.mean	
-LP.NPY : NA(10^{-5}M)	11.6 ± 3.0	
+LP.NPY : NA(10^{-5}M)	93.2 ± 11.9	*
" +Phen(10^{-7}M)	15.2 ± 3.5	*
" +Benxt(10^{-7}M)	58.2 ± 8.6	*
" +PYX1(10^{-7}M)	50.8 ± 14.2	*

Table1b: NA response ± [11-36]NPY (10^{-9}M)

Pretreatment	ΔPerf. Press. (mmHg) ± s.e.mean	
-[11-36]NPY: NA($3x10^{-5}$M)	44.2 ± 7.5	
+[11-36]NPY: NA($3x10^{-5}$M)	113.7 ±15.2	*
" +Phen(10^{-7}M)	23.0 ± 9.6	*
" +Benxt(10^{-7}M)	68.6 ± 6.5	ns
" +PYX1(10^{-7}M)	95.0 ± 9.9	

(n=5-7; ANOVA; *P<0.05)

In conclusion the Y1 and Y2 receptors may both be present at pre- and postsynaptic locations in this preparation. The observed potentiation of NA-stimulated increase in perfusion pressure mediated through these receptors may be differentially altered by alpha-adrenoceptor antagonists and the putative NPY antagonist PYX1.

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54P ENDOTHELIN-1 ATTENUATES THE VASODILATOR RESPONSES TO BRL-38227 AND Ro 31-6930, BUT NOT RP-49356 OR NITRENDIPINE, IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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Endothelin-1 (ET-1) decreases the vasorelaxant potency of BRL-38227 (BRL), but not of RP-49356 (RP) or pinacidil in rat aorta (Lawson *et al.*, 1990a), even at concentrations subthreshold for contraction (Lawson *et al.*, 1990b). We now report the inhibitory effects of ET-1 on the vasodepressor effects of the K$^+$-channel activators (PCAs) BRL, RP and Ro 31-6930 (Ro: Paciorek *et al.*, 1990) or nitrendipine (NT). In anaesthetised (pentobarbitone 50mg/kg, i.p.) SHRs (250-350g) cumulative vasodilator curves to BRL or Ro (1-1000μg/kg, i.v.), RP (10-1000μg/kg, i.v.) or NT (0.1-30μg/kg, i.v.) were made 4 min after ET-1 (1 or 10 μg/kg, i.v. bolus) or saline (1ml/kg); 15% (ED_{15}) and 50% (ED_{50}) falls in diastolic blood pressure (DBP) were calculated. In conscious SHRs (280-330g), with indwelling catheters to measure aortic DBP, BRL (30μg/kg) or RP (100μg/kg) were given orally 4 min after ET-1 (1μg/kg, i.a. bolus) or saline. Maximum fall in DBP (ΔDBP_{max}) and Areas Under the Curve (AUC) were calculated. Cumulative concentration-response curves (0.01-30μM) to BRL, RP and Ro were constructed in aortic rings contracted with either KCl or ET-1 and pIC_{50} values calculated (Lawson *et al* ., 1990a). Mean ± s.e.mean (n = 4-6) values for each treatment group are shown below:

Treatment	DBP (ED_{15} μg/kg) Vehicle	ET-1 (1μg/kg iv)	ET-1 (10μg/kg iv)	DBP ($AUC_{0-30min}$ %.min) Vehicle	ET-1 (1μg/kg ia)	ΔDBP_{max}(%) Vehicle	ET-1 (1μg/kg ia)	Aorta (pIC_{50}) KCl (20mM)	ET-1 (10nM)
BRL	8.2±2.2	13.2±0.7*	23.7±3.2*	482±71	221±40*	-24.8±4.0	-10.2±1.4*	7.6±0.1[a]	6.8±0.1[#a]
Ro	5.2±1.0	11.7±2.5*	-	-	-	-	-	7.8±0.1	6.2±0.2[#]
RP	53.3±7.0	55.0±6.0	-	384±75	266±79	-18.8±4.7	-17.0±3.5	6.5±0.1[a]	6.3±0.1[a]
NT	1.2±0.3	-	0.7±0.1	-	-	-	-	-	-

** and # = significantly different from saline or KCl, respectively p<0.05 (unpaired t-test); a from Lawson et al., 1990a.*

ET-1 evoked a transient fall in DBP (1μg/kg i.v. = 75±6mmHg, n =17; 10μg/kg i.v. = 77±6mmHg, n = 9; 1μg/kg i.a. = 29±9mmHg, n = 10), which returned to base-line levels within 4 min. ED_{15} values for BRL and Ro, but not RP or NT were significantly increased after ET-1 (1μg/kg), whilst ED_{50} values were not modified. After ET-1 (10μg/kg), BRL (1000μg/kg) failed to produce a 50% fall in DBP. In aorta, Ro and BRL were 40 and 6 times less potent, respectively, on ET-1 than KCl contractions, while RP showed equivalent potency. Thus, ET-1 selectively attenuated the hypotension to low doses of BRL-38227 and Ro 31-6930, but not RP-49356 or nitrendipine in SHRs. These data, supported by *in vitro* results from normotensive rat aorta, suggest that ET-1 inhibits the vasorelaxant mechanism of low doses of BRL and Ro. Elevated endogenous levels of ET-1 might radically modify cardiovascular effects of some, but not all, PCAs.

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55P COMPARISON OF THE EFFECTS OF VASODILATOR DRUGS ON INTRAOCULAR PRESSURE AND VASCULAR RELAXATION

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The isolated, arterially perfused bovine eye (Wilson, 1988) offers a quick and cheap assessment of the ability of drugs to lower intraocular pressure (IOP), giving dose-dependent responses to standard drugs and allowing simultaneous observation of effects on the vasculature. The present work investigates whether vasodilator drugs alter IOP directly or by means of vascular relaxation.

Bovine eyes were cannulated via a posterior ciliary artery, within 50 min of slaughter, as described by Millar et al. (1990). Drugs or saline control were applied by bolus injection into the arterial perfusate in volumes of 3 or 10µl. Initial IOP in control eyes rose very slowly during the perfusion, whereas each vasodilator drug caused a significant, sustained, dose-dependent decrease in IOP (Table 1, first part). Arterial perfusion pressure (30±5 mmHg) was not significantly altered by any of the drugs under these conditions of low vascular tone, suggesting that relaxation of resistance vessels is not involved in the fall in IOP. However, when vascular tone was increased by adding noradrenaline (10^{-5} M) to the perfusate, arterial perfusion pressure increased by 37±3 mmHg and dose-dependent relaxation to the same drugs was now observed (Table 1, last part).

Table 1.

	Dose	IOP (mm Hg)				Percent relaxation	
	nmol	start	end	Δ	n	of NA-induced tone	n
Nitro-	3	9.51±0.42	8.76±0.38**	0.75	10	38.89±6.74	9
prusside	10	8.37±0.94	7.41±0.79**	0.96	7	39.57±6.45	6
Azide	0.3	11.97±0.49	10.12±0.36**	1.85	7	24.20±4.54	19
	1	9.83±0.57	7.68±0.38**	2.15	7	47.03±10.12 †	9
Pinacidil	3	8.57±0.30	8.10±0.31	0.47	6	9.51±3.45	12
	10	8.73±0.78	7.75±0.55*	0.98	6	35.55±6.06 ††	12

Mean ± s.e.mean. *P<0.025, **P<0.001, start vs. end,
†P<0.025, ††P<0.001, high vs. low dose, Student's t-test.

The data confirm that three vasodilators with different mechanisms all appear to lower IOP, but little correlation appears to exist between the magnitude of IOP fall and that of vascular relaxation. Further work is continuing to establish whether the diverse mechanisms by which these compounds relax blood vessels also apply to their effects on aqueous humour dynamics.

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56P EVIDENCE THAT ACTIVATION OF α_2 ADRENOCEPTORS DOES NOT INHIBIT CALCIUM ENTRY INTO POSTGANGLIONIC SYMPATHETIC NERVE TERMINALS

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Studies on the cell bodies of various neurones have indicated that activation of α_2-adrenoceptors increase K^+ and decrease Ca^{2+} conductances (see Starke, 1987). Similar alterations in either K^+ and/or Ca^{2+} conductance have been suggested to play a role in the regulation of transmitter release from postganglionic sympathetic nerve terminals mediated through the activation of prejunctional α_2-adrenoceptors. However, to date there is no direct evidence supporting this contention. Focal extracellular recording techniques, which allow simultaneous recording of the nerve terminal impulse (NTI) and transmitter release, have shown that inhibition of transmitter release by the α-adrenoceptor agonist clonidine (0.1 µM) can occur without any detectable alteration in the NTI (Brock & Cunnane, 1991). However, this technique will tend to emphasize the high frequency components in the NTI and it is possible that alterations in slower conductances may be overlooked. In this inquiry the effects of clonidine on a slow component of the NTI revealed by the focal application of the K^+ channel blockers 4-aminopyridine (4-AP) and tetraethylammonium (TEA) have been investigated.

Focal extracellular recording techniques using a suction electrode (tip diameter ~50 µm) applied to the smooth muscle surface of the guinea-pig vas deferens *in vitro* were used (see Brock & Cunnane, 1988). All the nerve impulses recorded in this study were associated with transmitter release. However prior to studying the effects of drugs on the NTI, tissues were treated with α,β-methylene ATP (1 µM) to inhibit postjunctional electrical activity which may have masked delayed components of the NTI.

Perfusion of the recording electrode with a Hepes buffered solution containing 4-AP (1 mM) and TEA (10 mM) produced a late negative-going component (LNC) of the NTI. The LNC was unaffected by changing the Ca^{2+} concentration (0 - 10 mM) of the solution perfusing the electrode or by the local application of the N-type calcium channel blocker ω-conotoxin GVIA (CTX) (0.1 µM). Perfusion of the electrode with Cd^{2+} (0.1 - 0.3 mM) containing solution slightly reduced the amplitude and slowed the time course of the LNC in some experiments. The LNC was abolished when the bath was perfused with the K^+ entry blockers and was markedly reduced in amplitude and slowed in time course when Ca^{2+} was removed from the solution perfusing the bath. CTX (0.1 µM) produced similar effects to 'zero' calcium when added to the solution perfusing the bath. These findings suggest that the LNC is generated largely by the outward movement K^+ from sites outside the recording electrode. Furthermore, the sensitivity of this component of the NTI to the removal of Ca^{2+} or the addition of CTX suggest that a large component of the outward K^+ movement is through Ca^{2+} dependent K^+ channels. Bath application of clonidine (0.1 - 1 µM) little altered the LNC suggesting it does not alter Ca^{2+} conductance.

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57P EFFECTS OF CLONIDINE ON THE ACTION POTENTIAL IN CELL BODIES AND NERVE TERMINALS OF SYMPATHETIC NEURONES INNERVATING THE GUINEA-PIG VAS DEFERENS

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Many studies of the mechanism of action of drugs known to modify transmitter release through prejunctional receptors have been carried out on the cell bodies of neurones. The question arises whether drugs have different actions in the terminal regions of the same neurone (see Shen & Surprenant, 1990). The effects of the α_2-adrenoceptor agonist clonidine on action potentials and transmitter release were therefore studied in cell bodies and nerve terminals of the postganglionic sympathetic neurones innervating the guinea-pig vas deferens.

Intracellular recordings were made from cell bodies of postganglionic sympathetic neurones in the guinea-pig anterior pelvic ganglion *in vitro*. Cells projecting to the vas deferens were identified by the presence of antidromic action potentials when brief electrical stimuli (0.05 - 1 ms) were applied through rings electrodes positioned midway between the prostatic and epidydimal ends. Clonidine (1 - 100 μM) had no effects on action potentials evoked in the cell body by antidromic stimulation, intracellular current injection or preganglionic stimulation of the hypogastric nerve. Thus functional α_2-adrenoceptors are not present on the postganglionic cell body.

The effects of clonidine on the action potential and transmitter release from postganglionic sympathetic nerve terminals were also investigated using focal extracellular recording techniques (Brock & Cunnane, 1988) which allow simultaneous recording of the nerve terminal impulse (NTI), which is normally bi- or triphasic, and transmitter release. Local application of clonidine (0.1 - 1 μM) within the recording electrode powerfully inhibited transmitter release without any detectable alteration in the NTI as previously reported (Brock & Cunnane, 1991). However, bath application of clonidine (0.3 - 3 μM) in some, but not all, experiments resulted in a change in the configuration of the NTI and a reduction in transmitter release. In these experiments the electrode was perfused with either yohimbine (1 μM) or idazoxan (1 μM) in order to 'protect' the α-adrenoceptors located within the electrode. The effects on the NTI were characterized by a slowing of the negative-going component and a reduction in the peak-to-peak amplitude. In four experiments, bath application of clonidine resulted in local failure of impulse propagation in the terminals. The effects on the nerve impulse were, in some experiments at least, partially overcome by the bath application of yohimbine (1 μM) or idazoxan (1 - 3 μM).

These findings suggest that the nerve terminals and cell bodies of postganglionic sympathetic neurones respond differently to clonidine. In moderate concentrations (0.3 - 3 μM), clonidine modifies action potential propagation in the secretory terminals but not in the cell bodies of postganglionic sympathetic neurones by an as yet unidentified mechanism.

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J.A.B. and T.S. are Bristol-Myers Squibb Research Fellows

58P TOLERANCE DEVELOPS TO THE NEUROCHEMICAL CHANGES IN RAT BRAIN AFTER TREATMENT FOR 10 DAYS WITH THE SPECIFIC α_2-ADRENOCEPTOR ANTAGONIST, ATIPAMEZOLE

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The α_2-adrenoceptor antagonist, atipamezole (ATI) has been proposed as a drug treatment for Alzheimer's disease (Riekkinen Jr *et al.* , 1990). However, we know little of the long term effects of the drug, e.g. does both the receptor blockade and its neurochemical consequences remain intact or does tolerence develop?

Administration to female Wistar rats (145 - 205 g) of ATI twice daily for 10 days, 3 mg/kg by gavage did not cause any overt toxicity. No tolerance developed to the ability of ATI to antagonize the central effects of dexmedetomidine (100 μg/kg). This specific α_2-agonist which alone caused loss of righting reflex and a 10°C fall in the rectal temperature was completely antagonized in both chronically and acutely treated ATI rats. However, the normal neurochemical responses to ATI seen 4 h after a single dose of ATI, no longer were present when the rats were killed after the subchronic treatment (table 1). This was duplicated throughout the brain when it was subdivided into anatomically distinct regions.

Table 1 Changes in rat whole brain biogenic amines, their metabolites and tryptophan concentrations after a single dose (Acute) or subchronic treatment (Chron) with ATI 3 mg/kg, b.i.d. for 10 days

Treatment	5HIAA nmol/g	5-HT nmol/g	Trp nmol/g	DOPAC nmol/g	HVA nmol/g	DA nmol/g	MHPG-SO_4 nmol/g	NA nmol/g
Acute H_2O	1.84±0.09	2.40±0.15	22.88±0.85	0.66±0.07	0.44±0.03	3.86±0.14	0.39±0.11	1.76±0.03
Acute ATI	2.63±0.07**	2.59±0.09	30.26±0.97**	0.71±0.06	0.45±0.02	3.90±0.12	0.81±0.05**	1.53±0.08*
Chron H_2O	1.96±0.08	2.63±0.04	23.98±1.17	0.60±0.03	0.39±0.03	3.93±0.10	0.41±0.01	1.74±0.05
Chron ATI	1.91±0.09	2.80±0.02	24.79±1.66	0.54±0.02	0.35±0.01	3.83±0.11	0.50±0.02	1.75±0.06

Results are shown as means ± s.e. mean; n = 5; * = P<0.05; ** = P<0.01 Dunnet's test

In conclusion, tolerance developed to the central neurochemical effects of ATI administration within 10 days even though α_2-adrenoceptors in brain were still functionally blocked.

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59P THE INFLUENCE OF CALCIUM ON EXTRACELLULAR DOPAMINE LEVELS IN THE NUCLEUS ACCUMBENS

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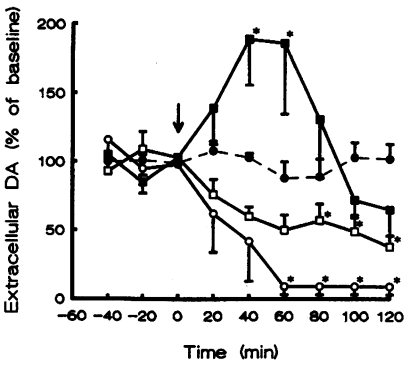

Figure 1: The influence of Ca^{++} on extracellular DA levels. At the point indicated by the arrow the Ca^{++} or Mg^{++} levels were changed as follows; 1.25 mM to 1.25mM Ca^{++} (-•-;control; N=4); 1.25mM to 0mM Ca^{++} (-▪-;N=7); 3.4mM to 0mM Ca^{++} (-□-;N=8); 1.25 mM Ca^{++} to 1.25mM Ca^{++} +12mM Mg^{++} (-o-;N=3). The results are means ± SEM. *p<0.05; significantly different from control (Duncan's test).

The removal of Ca^{++} from the Ringer solutions used for in vivo dialysis has been shown to cause substantial reductions in the recovery of dopamine (DA) from the extracellular (EC) space (Imperato & Di Chiara, 1984; Westerink et al., 1988). In these studies, however, the initial Ca^{++} concentrations in the Ringer have tended to be higher than the endogenous EC Ca^{++} levels (1.2mM) reported by Somjen et al., (1987). The present study examined the influence of the initial Ca^{++} concentration on the change in dialysate DA levels evoked by changing to a Ca^{++}-free solution.

Male Sprague-Dawley rats were anaesthetised with Avertin and dialysis loops implanted stereotaxically in the nucleus accumbens. Twenty four hours later, the probes were dialysed at 1.7µl per minute with Ringer solution containing either 1.25 or 3.4mM Ca^{++}. After a period of equilibration (40 minutes), 9 x 20 minute samples were collected and assayed for DA using HPLC with electrochemical detection. The dialysis solutions were switched to the solutions outlined in figure 1 after the collection of the third sample. As anticipated, switching from a Ringer solution containing 3.4mM Ca^{++} to a Ca^{++}-free solution significantly ($p<0.01$) reduced EC DA (Fig 1). In contrast, when the initial Ca^{++} concentration was 1.25mM, switching to the Ca^{++}-free solution caused an initial increase ($p<0.05$) in EC DA although the levels did eventually fall below controls. The addition of magnesium (Mg^{++}) to the Ringer solution caused an immediate and sustained fall ($p<0.001$) in dialysate DA levels. The results indicate that the effects on dialysate DA levels of switching to a Ca^{++}-free solution are influenced by the initial Ca^{++} concentration and suggest that Ca^{++} dependence is better demonstrated by using a Ca^{++} channel blocking agent such as Mg^{++}.

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60P THE NEW SELECTIVE D_1 DOPAMINE RECEPTOR AGONIST A-68930: PSYCHOPHARMACOLOGICAL CHARACTERISATION IN RELATION TO D_1:D_2 INTERACTIONS

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The great majority of our new notions on the psychopharmacology of D-1 dopamine (DA) receptor agonists derive from the benzazepine SK&F 38393 or close homologues thereof (Waddington & O'Boyle, 1989; Murray & Waddington, 1989). However, this agent shows incomplete selectivity, relatively poor penetration into the brain and modest, partial agonist activity to stimulate adenylate cyclase. It therefore appears essential to substantiate and extend these notions, including evidence for two directions of D-1:D-2 interaction regulating typical and atypical DAergic behaviours, using chemically distinct, selective D-1 agonists as they become available.

Using previously described procedures (Murray & Waddington, 1989; Daly & Waddington, 1991), rats were challenged with the new, selective D-1 agonist A-68930 (DeNinno et al., 1990) alone, or after pretreatment with the selective D-1 antagonist SCH 23390 or the selective D-2 antagonist YM 09151 (Waddington & O'Boyle, 1989), and behavioural responses assessed; the selectivity of A-68930 for D-1 vs D-2 receptors was clarified in binding studies using ^{3}H-SCH 23390 and ^{3}H-spiperone. A-68930 (0.0625-4.0 mg/kg) potently induced intense grooming ($P<0.01$ at 0.25 mg/kg), with modest induction of vacuous chewing ($P<0.05$ at 0.25 mg/kg); sniffing but not locomotion was also evident, particularly at higher doses ($P<0.01$ at 4.0 mg/kg). Typical intense grooming to A-68930 (0.25 mg/kg) was readily antagonised ($P<0.01$) by 0.01-1.0 mg/kg SCH 23390, and also by 0.005-0.5 mg/kg YM 09151 in accordance with a permissive role for D-2 tone via cooperative D-1:D-2 interactions. However, vacuous chewing to A-68930 was not readily antagonised by SCH 23390 and appeared somewhat enhanced ($P=0.05$) by YM 09151. A-68930 showed >200-fold selectivity for D-1 vs D-2 receptors.

These studies indicate that A-68930 shares the general psychopharmacological profile of SK&F 38393; it induces typical intense grooming much more potently and robustly than does SK&F 38393, under apparent regulation by cooperative D-1:D-2 interactions. A-68930 also induces some vacuous chewing, a more controversial behavioural model of D-1 receptor activation; however, this atypical response appeared to involve either a D-1-like receptor relatively insensitive to SCH 23390 but regulated by oppositional D-1:D-2 interactions, or else some alternative mechanism(s) that remain to be defined.

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61P CONTRAVERSIVE TURNING INDUCED BY THE DOPAMINE D_1 AGONIST SKF 89 626 IN UNILATERALLY SUBSTANTIA NIGRA LESIONED RATS

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Brain dopamine receptors are subdivided into the adenylate cyclase linked D-1 site and the D-2 receptor sites some of which are negatively linked to this enzyme. It is well established that stimulation of D-2 receptors is the active principle of antiparkinsonian and antihyperprolactinaemic agents. The functional role of D-1 receptor stimulation is less clear. The D-1 agonist studied most intensively in animals is the benzazepine derivative SKF 38 393. This compound has no antiparkinsonian activity and elevates plasma prolactin in humans. However, being only a partial agonist at D-1 receptors, SKF 38 393 is not the optimal tool. We have therefore tested a novel D-1 agonist the thienopyridine derivative SKF 89 626 for it's ability to induce contralateral rotations in unilaterally substantia nigra (SN) lesioned rats.
6-OHDA (16µg/4µl saline) was injected into the left SN of male Wistar rats 2-4 months prior to the experiments. Rotations were registered by means of an automatic device consisting of a perspex bowl and a mechanical transducer system. SKF 89 626 was given either alone (1.56, 6.25 and 25 mg/kg i.p.) or in a fixed dose (6.25 mg/kg i.p.) combined with SCH 23 390 (0.39, 1.56 and 6.25 mg/kg i.p.) or raclopride (0.1, 0.39 and 1.56 mg/kg i.p.). SKF 38 393 (0.39, 1.56 and 6.25 mg/kg i.p.) served as reference compound. Individual ipsiversive and contraversive rotations were accumulated over 60 min in groups of 7-8 animals as 36° turns (counts in table 1).

Table 1: Contraversive rotations in unilaterally SN lesioned rats

Mean counts per 60 min (SEM, N = 7-8, * p<0.05, ** p<0.01, Dunnett test)

	Vehicle	0.1	0.39	1.560	6.25	25.0 mg/kg ip
SKF 38 393	441 (75)		489 (61)	1401 (568)	1897 (653) *	
SKF 89 626	523 (86)			1041 (149)	2134 (514)	5341 (1356) **
SKF 89 626 (6.25 mg/kg i.p.)						
+ SCH 23 390	4427 (804)		1117 (296) **	1084 (258) **	1462 (412) **	
+ Raclopride	3556 (668)	3853 (713)	4129 (538)	2113 (493)		

These results indicate, that SKF 89 626 is a centrally active D-1 receptor agonist since its activity is antagonized by the D-1 antagonist SCH 23 390 but not by the D-2 antagonist raclopride. The lower potency of the compound when compared to SKF 38 393 might reflect a poorer central bioavailabilty.

62P THE EFFECT OF SODIUM VALPROATE ON DOPAMINE AND 3,4-DIHYDROXYPHENYLACETIC ACID LEVELS IN RAT HIPPOCAMPAL DIALYSATES *IN VIVO*

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Sodium Valproate (NaVPA) is a widely used anti-convulsant agent with indications for most epilepsies. NaVPA is thought to augment the action of GABA in the CNS, although its mode of action remains uncertain. Whitton *et al.* 1985 reported NaVPA-induced increases in 5-hydroxytryptamine synthesis, whilst that of whole brain dopamine (DA) was unaffected by the drug (Whitton, unpublished data).

The aim of this study was to examine the effects of NaVPA on extracellular levels of DA and its metabolites in the rat ventral hippocampus, employing *in vivo* microdialysis.

Male Wistar rats (280-380g) were anaesthetised with chloral hydrate (400mg/ml) and stereotaxically implanted with concentric microdialysis probes into the ventral hippocampus. Perfusion with artifical cerebrospinal fluid, containing 1µM citalopram, at a rate of 0.5µl/min, was commenced 18h postoperatively and dialysates collected every 30 mins following an initial 30 min stabilisation period. Five consecutive samples were collected prior to an intraperitoneal injection of NaVPA (100-400mg/kg) or saline. Sample dialysates were analysed using HPLC with electrochemical detection. Basal concentrations of DA and DOPAC were found to be 9.35 ± 1.1 and 58.2 ± 5.3nM respectively (mean ± s.e.mean, n=20).

The results clearly indicate that NaVPA dose-dependently increased extracellular levels of DA and DOPAC, with the most marked effects seen at 400mg/kg NaVPA (278 ± 65%, n=20 and 1426 ± 462%, n=20 of mean basal levels respectively; see Figure 1 for DA data). In contrast extracellular levels of the DA metabolite, HVA were unchanged in the presence of NaVPA. NaVPA treatment induces dose-dependent increases in dialysate DA and DOPAC, but not HVA levels by means of an, as yet, uncharacterised mechanism. Future work aims to assess the effects of chronic low dose NaVPA treatments upon monoamines and amino acid transmitters in various brain regions, possibly by infusing NaVPA directly into the area of interest via the probe.

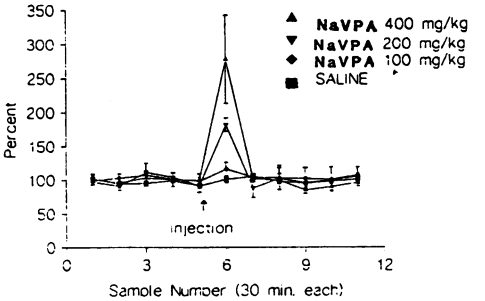

Figure 1.

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63P THE ANGIOTENSIN RECEPTOR ANTAGONISTS DuP 753 AND WL 19 BLOCK APOMORPHINE-INDUCED STEREOTYPY IN THE RAT

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Recent data suggests that a functional interaction exists between the brain angiotensin system and dopaminergic neurotransmission. Thus, angiotensin II injected intracerebroventricularly potentiates apomorphine-induced stereotyped behaviour (Georgiev *et al*., 1985), whereas the angiotensin converting enzyme (ACE) inhibitors captopril and enalapril block apomorphine-induced oral stereotypy (Banks *et al*., 1990). Angiotensin receptors have been classified into two subtypes which are present in both brain and peripheral tissues. The receptor subtypes can be discriminated on the basis of showing high affinity for either DuP 753 (AT_1) or WL 19 (AT_2) both of which are novel non-peptide angiotensin receptor antagonists (Chang & Lotti, 1990). We have now assessed the effects of DuP 753 and WL 19 on apomorphine-induced stereotyped behaviour in the rat.

Male Sprague Dawley rats (180-220g) were habituated to Perspex observation boxes for 45 min and injected s.c. with DuP 753 (10, 30 or 100 mg/kg), WL 19 (3, 10, 30 or 100 mg/kg) or vehicle (0.5% Methocel in distilled water). The rats were returned to the boxes for 50 minutes and then injected s.c. with 1 mg/kg apomorphine. The duration of licking and gnawing and the frequency of cage crossing were recorded for 10 min (beginning 10 min after the second injection), using a keypad linked to a BBC microcomputer. In addition, at the 100 mg/kg dose of DuP 753 and the 30 mg/kg dose of WL 19, the frequency and duration of vacuous mouth movements, penile grooming, immobility, and sniffing, and frequency of yawning were recorded.

DuP 753 at a dose of 30 mg/kg blocked apomorphine-induced gnawing, but had no effect on apomorphine-induced licking or locomotor activity. A dose of 100 mg/kg DuP 753 blocked apomorphine induced gnawing, licking, and sniffing. In addition, the combination of DuP 753 (100 mg/kg) and apomorphine produced immobility, vacuous mouth movements, penile grooming and yawning. DuP 753 at a dose of 10 mg/kg had no effect on the apomorphine response. WL 19 at a dose of 10 mg/kg blocked apomorphine-induced gnawing and potentiated licking but had no effect on locomotor activity. A dose of 30 mg/kg WL 19 attenuated apomorphine-induced sniffing, and licking, and blocked gnawing. Furthermore, the combination of 30 mg/kg WL 19 and apomorphine induced yawning and associated behaviours, as described above with DuP 753. WL 19 (3 and 100 mg/kg) had no effect on apomorphine-induced behaviour.

These data suggest that the blockade of apomorphine-induced stereotypy by ACE inhibitors, observed in a previous study (Banks *et al*., 1990) is mediated by an action on angiotensin receptors. The appearance of the yawning syndrome after injection of 100 mg/kg DuP 753 or 30 mg/kg WL 19 with 1 mg/kg apomorphine suggests that the antagonists produced a 10 fold shift to the right of the apomorphine dose response curve as 0.1 mg/kg is the maximal dose to induce yawning in rats given apomorphine alone. Since both DuP 753 and WL 19 were equally effective (only a 3 fold difference in potency) in blocking apomorphine-induced stereotypy in the present study, the results suggest that both AT_1 and AT_2 receptors are involved in mediating angiotensin/dopamine interactions.

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64P 5-HT_1-LIKE AND 5-HT_{1B} RECEPTOR LIGANDS BUT NOT 5-HT_{1A} LIGANDS MODULATE 5-HT RELEASE IN THE SUPRACHIASMATIC NUCLEUS OF THE RAT

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Fast cyclic voltammetry (FCV) can measure changes in stimulated endogenous 5-HT overflow ([5-HT]ex) from untreated rat brain slices (O'Connor and Kruk, 1991a). As changes in [5-HT]ex cannot be elicited reproducibly by a single electrical pulse, we have used pseudo single pulse (PSP) stimulation (5 pulses at 100Hz; Singer, 1988; O'Connor and Kruk, 1991b).

[5-HT]ex was monitored with a carbon fibre microelectrode positioned 100µm from a bipolar stimulating electrode (tip separation 200µm) and placed centrally in a 350µm thick rat brain slice incorporating the suprachiasmatic nucleus (SCN). PSP stimulation was applied every 5min and reproducible [5-HT]ex (10 to 15nM 5-HT) could be measured for more than 6h (O'Connor and Kruk, 1991a).

Figure 1 shows the effects of putative 5-HT_1-like receptor ligands (5-carboxamidotryptamine (5-CT) and GR43175 (Humphrey et al., 1988), 5-HT_{1A} ligands 8-OHDPAT and ipsapirone and the 5-$HT_{1A/1B}$ ligand, RU24969; (Tricklebank et al., 1986) on [5-HT]ex in the SCN. The 5-HT_{1A} selective ligands 8-OH-DPAT and ipsapirone had no effect on [5-HT]ex up to 10^{-6}M. By contrast the 5-HT_1-like agonists (5-CT and GR43175) and the 5-$HT_{1A/1B}$ ligand RU24969 caused a dose-dependent inhibition of endogenous 5-HT overflow; ED_{50} values were 5.6, 39.5 and 3.8nM respectively. These results show that 5-HT_1-like agonists may interact with 5-HT_{1B} sites at axon terminals which act as 5-HT autoreceptors; 5-HT_{1A} ligands at up to 10^{-6}M do not interact with functional 5-HT autoreceptors at axon terminals. These results are in accord with studies *in vivo* (Marsden & Martin, 1985).

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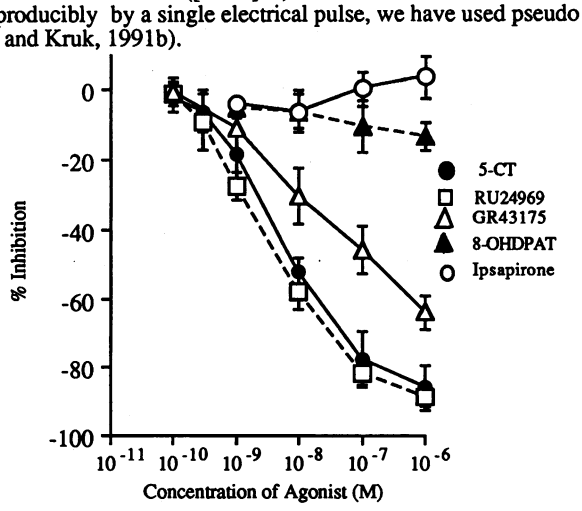

Figure 1 Effects of 5-HT receptor ligands (indicated) on [5-HT]ex. n=3-6 for all points.

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65P EVIDENCE TO SUGGEST THAT ACTIVATION OF FOREBRAIN 5-HT$_1$ RECEPTORS CAUSES SYMPATHOEXCITATION IN ANAESTHETIZED RATS

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Lateral ventricular application of 5-HT causes a rise in blood pressure in the anaesthetized rat which may be due to activation of 5-HT receptors located in the anterior hypothalamus/preoptic region (see Coote, 1990). The nature of the 5-HT receptors which mediate this pressor effect were investigated.

In male Sprague-Dawley rats (250-375g) anaesthesia was induced with halothane and maintained with α-chloralose (80 mg kg^{-1}). Rats were artificially ventilated following neuromuscular blockade with decamethonium iodide (1 mg per animal). Simultaneous recordings were made of blood pressure (BP), heart rate (HR), renal (RNA) and phrenic nerve activity (PNA). 5-HT (n=6-8; 40, 120 nmol kg^{-1}), 5-CT (n=5; 3 nmol kg^{-1}), DP-5-CT (n=4-7; 0.3, 3 nmol kg^{-1}), 8-OH-DPAT (n=8-9; 40, 120 nmol kg^{-1}) and saline (n=6) were given by microinjection (5 µl over 20s) into the right lateral ventricle (i.c.v.). In separate experiments methiothepin (n=4; 1 mg kg^{-1}) was given i.v. 20 min before DP-5-CT.

5-HT, DP-5-CT and 5-CT caused rises in BP, HR and RNA of 24 ± 3 mmHg, 63 ± 13 beats min^{-1}, 126 ± 33 % and 24 ± 4 mmHg, 63 ± 9 beats min^{-1}, 112 ± 29 % for the highest dose of 5-HT and DP-5-CT respectively, and 14 ± 4 mmHg, 42 ± 4 beats min^{-1}, 142 ± 43 % for 5-CT. 8-OH-DPAT 40 and 120 nmol kg^{-1} only caused a rise in HR, of 28±4 and 50 ± 13 beats min^{-1} respectively. 5-HT differed from DP-5-CT and 5-CT in that the rise in blood pressure was associated with an initial decrease in HR and RNA. 8-OH-DPAT also differed in that it was the only compound to consistently increase PNA. Pretreatment with methiothepin attenuated the effects of DP-5-CT on all variables.

These results suggest that activation of 5-HT$_1$ receptors are responsible for the sympathoexcitation and pressor effects observed when 5-HT is injected into the lateral ventricle in anaesthetized rats.

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66P 5-HT$_{1A}$ AND NMDA BUT NOT 5-HT$_2$ RECEPTORS ARE ENRICHED ON RAT NEOCORTICAL PYRAMIDAL NEURONES SELECTIVELY DESTROYED BY INTRASTRIATAL VOLKENSIN

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Striatal injections of 6ng volkensin may selectively destroy a sub-population of neocortical pyramidal neurones (27% loss; Pangalos et al, 1990). The selectivity of the lesion is now confirmed by the preservation of glutamic acid decarboxylase mRNA positive cells (GAD), considered to be interneurones, by in situ hybridization histochemistry (Najlerahim et al, 1990). The number of GAD cells per field (mean ± SEM, n=5) was not significantly different (Student's t test) in sections ipsilateral to the lesion (lower cortical layers, 3.75 ± 0.03) vs. those contralateraly (3.93 ± 0.15). With 2ng volkensin a similar reduction in the number of pyramidal neurones (29%) with preservation of GAD was observed. Receptor density in cryostat sections (2ng volkensin) was determined using 1nM [^{3}H]-8-OH-DPAT for the 5-HT $_{1A}$ subtype (Pazos and Palacios, 1985) and 2nM [^{3}H]-ketanserin for the 5-HT$_2$ subtype (Pazos et al 1985). For the NMDA receptor sections were preincubated (1 h in 50mM Tris-acetate, pH 7, 4°C), incubated in fresh buffer (2 hours, 4°C) containing 5nM [^{3}H] L-689,560, a selective glycine site antagonist (Grimwood et al 1991), and washed in buffer (4x1 min, 4°C). Non-specific binding (10-20% of total) was defined using 1mM glycine. Binding density of ligands was quantified by image analysis using calibrated [^{3}H] micro-scales. The results (Table 1) suggest that the sub-population of cortical pyramidal neurones lost are enriched more in 5HT $_{1A}$ receptors than in the NMDA receptor complex. A potential marker of interneurones, [^{3}H]-ketanserin binding, was not affected, consistent with the preservation of GAD.

Table 1. Binding density in lower cortical layers (mean ± SEM, fmol/mg tissue, n=7, 3-8 sections from each rat)

*P<0.05, **P<0.01 vs. contralateral paired t test.		[^{3}H]-8-OH-DPAT	[^{3}H] L-689,560	[^{3}H]-ketanserin
	contralateral	31.8 ± 7.1	275 ± 25	32.7 ± 3.1
	ipsilateral	25.9 ± 6.8**	246 ± 31*	28.2 ± 2.1

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67P OPPOSING ROLES FOR 5-HT$_1$ AND 5-HT$_3$ RECEPTORS IN CONTROLLING 5-HT RELEASE IN RAT HIPPOCAMPUS *IN VIVO*

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Stimulation of 5-HT3 receptors has been shown to modulate neurotransmitter release in the cortex, i.e. inhibit acetylcholine in rat (Barnes et al., 1989) and enhance 5-hydroxytryptamine (5-HT) in guinea pig (Galzin et al., 1990). However, 5-HT3 receptors are known to occur in relatively high density in other limbic areas such as hippocampus (Kilpatrick et al., 1987) and, consequently, we have employed in vivo microdialysis in the rat to determine whether these receptors are also implicated in the control of 5-HT release in this region.

Concentric microdialysis probes (Carnegie Medecin) were stereotaxically implanted into the ventral hippocampus of chloral hydrate (600 mg/kg ip and 30 mg as required) anaesthetised male Wistar rats (280 - 300 g; Olac, Bicester). The probes were perfused (1 µl/min) with an artificial cerebrospinal fluid containing the selective 5-HT reuptake inhibitor citalopram (1 µM). Perfusates were collected every 15 min and analysed for 5-HT by HPLC-ECD. After a 2 h stabilisation period, drugs (1 µM) were administered via the perfusion fluid in a 15 min pulse. Basal release of 5-HT was 22.4 ± 1.5 fmoles per 15 min sample (n = 24). As shown in Table 1, the 5-HT1 agonist, RU 24969, decreased dialysate 5-HT levels by 47%. By contrast, the selective 5-HT3 agonist, 2-methyl-5-HT (2Me5-HT) increased 5-HT output by 43% ; an effect which was abolished by the 5-HT3 antagonist MDL 72222 at a concentration that did not markedly alter the release of 5-HT when infused alone .

Table 1 The effect of 5-HT receptor agonists and antagonists on 5-HT release in the ventral hippocampus of the rat

DRUG	n	0 min	15 min	30 min	45 min	60 min
Control	6	100	107 ± 6	98 ± 6	103 ± 9	98 ± 5
RU 24969	5	100	74 ± 7*	53 ± 18*	58 ± 8*	87 ± 4
2Me5-HT	4	100	143 ± 3*	130 ± 15	125 ± 8	125 ± 12*
MDL 72222	4	100	101 ± 17	77 ± 15	117 ± 19	111 ± 18
MDL 72222 + 2Me5-HT	5	100	105 ± 7	94 ± 15	92 ± 21	92 ± 17

Values are mean ± s.e.mean percentage of dialysate 5-HT concentration at time 0 min. *P < 0.05 Dunnett's test versus control.

As previously reported by Sharp et al. (1989), we observed that the 5-HT1B autoreceptor agonist, RU 24969, inhibits 5-HT release in the hippocampus. Moreover, we have demonstrated that 5-HT3 receptor stimulation in this 5-HT-containing nerve terminal area potentiates release of this neurotransmitter indicating an opposing role for these receptors in controlling 5-HT function. Therefore, these data argue that presynaptic, facilitatory 5-HT3 receptors are involved in the control of 5-HT release, in addition to the classical 5-HT1B autoreceptor.

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68P (+)-TUBOCURARINE AND COCAINE REVEAL SPECIES DIFFERENCES IN THE 5-HT$_3$ RECEPTORS OF RABBIT, MOUSE AND GUINEA-PIG NODOSE GANGLION NEURONES

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Low nanomolar concentrations of (+)-tubocurarine [(+)-Tc] are effective in antagonizing the 5-HT$_3$ receptors expressed by mouse hippocampal neurones and the neuronal cell lines N1E-115 and NG108-15 (Yakel & Jackson, 1988; Peters et al., 1990). However, 5-HT$_3$ receptor mediated depolarizations of guinea-pig submucous plexus and coeliac ganglion neurones are far less sensitive to this antagonist (Vanner & Suprenant, 1990; Wallis & Dun, 1988). It is not clear whether the differential sensitivity to (+)-Tc is dependent upon the tissue, the species or both. In this study we have compared the antagonist actions of (+)-Tc and cocaine upon the 5-HT$_3$ receptor mediated currents recorded from voltage-clamped adult rabbit, mouse and guinea-pig nodose ganglion neurones (Peters et al., 1991).

Patch electrode techniques, in conjunction with either List L/M EPC-7 or Axoclamp 2A recording systems, were employed to voltage-clamp isolated nodose ganglion neurones maintained in culture for 1-7 days. Recordings were made at a holding potential of -60mV and at 17-23°C. The concentration of antagonist required to inhibit the 5-HT-induced current to 50% of control (IC_{50}) was determined from dose-response curves constructed from 3-16 cells.

In rabbit, mouse and guinea-pig nodose ganglion neurones (NGN's), locally applied 5-HT (10µM) induced an inward current associated with a conductance increase which was abolished by 1nM, 3nM and 300nM ondansetron respectively. These observations suggest the involvement of 5-HT$_3$ receptors. (+)-Tc antagonised the 5-HT-induced current in a concentration dependent, fully reversible manner, producing IC_{50} values of 1.6nM, 170nM and 9µM in mouse, rabbit and guinea-pig NGN's respectively. In rabbit NGN's, the inhibition produced by 300nM (+)-Tc was voltage independent over the range tested (-80mV to +40mV). The 5-HT$_3$ antagonist potency of cocaine also demonstrated a differential sensitivity with IC_{50} values of 80nM, 2µM and 4.6µM in rabbit, guinea-pig and mouse NGN's respectively. Hence, cocaine exhibits a different rank order of potency than that seen with (+)-Tc.

These data, therefore, demonstrate that the differences observed in 5-HT$_3$ antagonist potency of (+)-Tc and cocaine in NGN's of mouse, rabbit and guinea-pig can be attributed to species differences in the 5-HT$_3$ receptor, rather than anatomical location. Whether there are tissue differences in 5-HT$_3$ receptor properties within a species remains to be determined.

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69P EFFECT OF DIAZEPAM ON EXTRACELLULAR 5-HT IN THE VENTRAL HIPPOCAMPUS OBSERVED IN RATS ON THE X-MAZE MODEL OF ANXIETY USING *IN VIVO* MICRODIALYSIS

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It has been suggested that one of the mechanisms of the anxiolytic effects of benzodiazepines is via a decrease in central serotonergic transmission (Stein et al., 1977). Further evidence has shown that benzodiazepines inhibit 5-HT synthesis (Saner and Pletscher, 1979) and release (Pei et al., 1989). The aim of this study was to investigate changes in extracellular 5-HT, using intracerebral microdialysis, during exposure to the elevated X-maze, a model of anxiety (Handley and Mithani, 1984), and to determine the effect of diazepam (DZP) on extracellular 5-HT under these conditions.

Male Lister hooded rats (250-300g) were anaesthetised with halothane (1.5-2%) and a microdialysis probe was stereotaxically implanted in the ventral hippocampus. The animals were allowed to recover for 24 hrs. and then perfused with artificial CSF at a flow rate of 1µl/min. 30 min after the start of perfusion baseline measurements of extracellular 5-HT were made for 80 min with samples collected every 20 min. Animals then received an injection of either vehicle (1% methyl cellulose) or DZP (2.5 mg/kg) i.p. and 40 min later placed on the X-maze (facing one of the enclosed arms) and behaviour monitored for 20 min in 5 min periods during which time dialysis continued. After this animals were returned to their home cages and dialysis samples collected every 20 min for a further 2 hrs. Measurement of 5-HT in the dialysis samples was by HPLC with electrochemical detection.

Placing the animals on the X-maze for a 20 min period increased the levels of extracellular 5-HT by 101±25.9% (n=6) above basal in the control animals. Extracellular 5-HT returned to basal level 40 min after control animals were replaced in their home cages. DZP (2.5 mg/kg) produced a significant anxiolytic effect on the X-maze (increase in time spent on the open and end of the open arms and in the % open:total entries and time) (2-tailed Mann-Whitney U-test) and significantly reduced the increase in extracellular 5-HT observed when the animals were placed on the X-maze compared to vehicle controls (Vehicle: 101±25.9%, DZP: 9±10.2%, n=6, $p<0.05$, one-way ANOVA with post-hoc Dunnett's t-test). Furthermore, DZP produced a significant decrease in extracellular 5-HT (50±6.9% of vehicle control; $p<0.01$) 100 min after removal from the X-maze.

The present study demonstrates that placing rats in the X-maze results in raised extracellular 5-HT in the ventral hippocampus, indicating increased release, and that the anxiolytic effects of DZP may be associated with an attenuation of the increase in 5-HT release.

I.K.W. is a SERC CASE student in conjunction with SmithKline Beecham.

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70P EFFECT OF EXTERNAL AND INTERNAL pH ON GABA-ACTIVATED CHLORIDE CONDUCTANCE AND ITS DESENSITISATION IN CRAYFISH MUSCLE FIBRES

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We have used a three-electrode voltage clamp and H^+-selective microelectrodes to examine the effect of intracellular, extracellular and surface pH, (pH_i, pH_o and pH_s) on the GABA-activated Cl^- conductance (G_{GABA}) in the crayfish leg opener muscle fibre. Fig.1 illustrates that at a higher pH_o the peak value of G_{GABA} is lower and it shows a more prominent desensitization. These effects were observed in the pH_o range, 6.4-8.4. Similarly, altering pH_s by application and withdrawal of weak acids and bases gave rise to transient changes in G_{GABA}, which were also inversely related to, and paralleled the changes in pH_s. Changes in pH_i (produced by the above-mentioned manoeuvres) between 6.6 and 8.0, however, did not have any detectable effect on the GABA-gated conductance.

A decrease of steady-state G_{GABA} produced by an increase in pH_o has been reported previously (Takeuchi & Takeuchi, 1967; Smart & Constanti, 1982). The present results suggest that in the presence of HCO_3^-, the transient increase in pH_s caused by the GABA-gated net efflux of HCO_3^- (Kaila et al. 1990) will induce a decrease in the peak value of the underlying conductance.

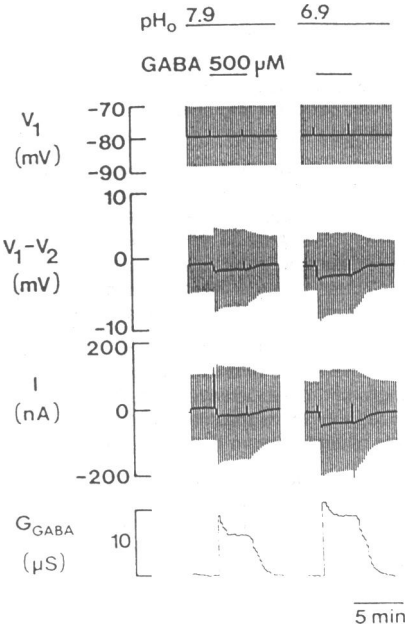

Fig.1. Effect of pH_o on G_{GABA}. The voltage clamp is imposed on the electrode (V_1) lying in the centre of the fibre, adjacent to the current passing electrode. The second electrode (V_2) is placed midway between the centre and the end. G_{GABA} is calculated on line by a microcomputer.

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71P [^{3}H]-GABAPENTIN BINDS TO A NOVEL SITE IN RAT CORTICAL SYNAPTIC PLASMA MEMBRANES

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Gabapentin (1-(aminomethyl)cyclohexaneaceticacid) is a novel anticonvulsant with an unknown mechanism of action. Recent electrophysiological studies in cultured neurones have shown that the inhibition of NMDA-induced currents by high concentrations of Gabapentin was reversed by the addition of glycine or D-serine (Sprosen, 1990). Similarly, D-serine reversed the inhibition of pentylenetetrazol-induced seizures by Gabapentin (Oles et al, 1990), suggesting that Gabapentin may act at the glycine modulatory site of the NMDA receptor complex. To further investigate this possibility, we have developed an *in vitro* assay for measuring [^{3}H]-Gabapentin binding in brain membranes, and present here a preliminary account of the characteristics of this novel binding site.

Partially purified rat cortical synaptic plasma membranes (0.1-0.3 mg protein) were incubated with [^{3}H]-Gabapentin (specific activity 73 Ci/mmol) in 10mM HEPES buffer (pH 7.4) for 30 minutes at room temperature, before filtering under vacuum onto GFB filters and washing with 100mM NaCl. Non-specific binding, generally 20% of total binding (12000-15000 dpm using 20nM [^{3}H]-Gabapentin), was defined by 100μM Gabapentin. The results shown here represent the geometric mean (and range) of at least three separate determinations.

[^{3}H]-Gabapentin binding was saturable, reversible and reached equilibrium within 30 minutes. Scatchard analysis yielded curvilinear plots, indicating the presence of two binding sites or affinity states. The K_D for the higher affinity site was approximately 0.1μM, a value agreeing closely with the IC_{50} value (0.14μM, range 0.10-0.29μM) for the inhibition of [^{3}H]-Gabapentin binding by Gabapentin itself. Kinetic studies however, implied the existence of even higher affinity sites. [^{3}H]-Gabapentin (20nM) binding was potently displaced by a range of Gabapentin analogues, and by 3-substituted GABA derivatives (Andruszkiewicz and Silverman, 1990) previously shown to possess anticonvulsant activity (Silverman, personal communication). Thus, (R,S)-isobutyl GABA, (R,S)-n-butyl-GABA, (R,S)-cyclohexyl-GABA and 3-methyl GABA inhibited [^{3}H]-Gabapentin binding with IC_{50} values of 0.1 (0.08-0.11) μM, 0.8 (0.6-1.2) μM, 1.6 (1.2-2.6) μM and 13 (9.0-16) μM respectively. In contrast, GABA and baclofen were much less active at inhibiting [^{3}H]-Gabapentin binding, with IC_{50} values of 610 (440-770) μM and 850 (450-1740) μM respectively. Other anticonvulsants, e.g. carbamazepine, diazepam and valproate, only weakly displaced binding (IC_{50} values greater than 1mM). The polyamines spermine and spermidine displaced [^{3}H]-Gabapentin binding with IC_{50} values of 12 (8.1-15) μM and 15 (14-15) μM respectively. However, maximum inhibition observed was approximately 50% of the total specific binding as defined by 100μM Gabapentin. This pattern of inhibition was mimicked by magnesium chloride (IC_{50} value 27μM, range 16-46μM), suggesting that under the conditions of this assay, the polyamines and magnesium may share a common binding site. Glutamate, NMDA, glycine and D-serine only weakly inhibited [^{3}H]-Gabapentin binding, with IC_{50} values of greater than 1mM. Moreover, out of a wide range of other neuroactive compounds, only glutamine was found to potently inhibit [^{3}H]-Gabapentin binding (IC_{50} value 20μM, range 10-28μM).

In summary, we have described a novel and specific binding site, labelled with [^{3}H]-Gabapentin, which is sensitive to inhibition by 3-substituted GABA derivatives, Gabapentin analogues and glutamine. The polyamines and magnesium, but not other ligands acting at the NMDA/glycine receptor complex, also recognise this binding site with relatively high affinity.

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72P AUTORADIOGRAPHICAL DETECTION OF [^{3}H]-GABAPENTIN BINDING SITES IN RODENT BRAIN

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High affinity binding of the of the anticonvulsant Gabapentin to homogenates of rat cerebral cortex has been recently demonstrated (Suman-Chauhan et al, this meeting). Preliminary studies using homogenates prepared from separate regions of rat brain suggested that the binding site may have an heterogeneous distribution. Moreover little or no specific binding was detected in a range of peripheral tissues including the heart, lung, kidney, liver, pancreas and spleen. In order to examine in greater detail the localization of this novel and hitherto uncharacterised central binding site, we have measured the autoradiographical distribution of [3H]-Gabapentin binding sites in sections of rat brain.

Sections (10μm) of rat brain were thaw mounted onto gelatine-subbed slides allowed to dry and stored frozen at -20C. On the day of use the sections were thawed, dried and preincubated for 30min in 10mM HEPES (pH 7.4 at 20C) before being transferred to fresh incubation medium containing 40nM [^{3}H]-Gabapentin with or without 100μM unlabelled Gabapentin or 10μM 3-butyl-GABA to define non-specific binding which was equivalent in each case. After 30 minutes the sections were washed four times for 2 minutes each in ice-cold sodium chloride (100mM) before being dipped in deionised water to remove salt. Under these conditions specific [^{3}H]-Gabapentin binding represented between 60 and 70% of total binding. Shorter or longer wash times did not significantly alter the level of specific binding. The dried sections were apposed to tritium-sensitive film for 3 weeks to generate the autoradiographs which were analysed densitometrically using a Quantimet 920 image analyser.

[^{3}H]-Gabapentin binding sites were unevenly localised throughout the rat brain with the highest levels being measured in the cerebral cortex and particularly the outer layers of the frontal, parietal, occipital and entorhinal cortex. Lower levels were detected in subcortical structures including the basal ganglia and the substantia nigra, the hypothalamus and also the molecular layer of the cerebellum. Binding in the hippocampus was clearly organised with the highest levels being associated with the molecular layer of the dentate gyrus followed by the CA1 stratum radiatum. Very low levels of binding were detected in the cell body layers of the hippocampus and the white matter.

The failure of a wide range of putative neurotransmitters to influence homogenate binding suggests that the site may not be directly linked with any of the recognised receptor subtypes. Nonetheless the distribution of [^{3}H]-Gabapentin binding was remarkably similar to that described for certain excitatory amino acid receptors. This and the influence on binding of certain polyamines known to be present in the brain suggest, at least the possibility of an association between the binding site for this anticonvulsant and the excitatory amino acid system.

Suman-Chauhan, N., Hill D.R. and Woodruff G.N. (This meeting)

73P SYNERGISM OF THE AMPA-ANTAGONIST NBQX WITH L-DOPA IN THE MPTP-TREATED COMMON MARMOSET AND THE UNILATERALLY SUBSTANTIA NIGRA LESIONED RAT

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Based on recent evidence suggesting an abnormal activity of the subthalamic nucleus and its glutamatergic basal ganglia output projections in the MPTP-treated primate (1), beneficial effects of NMDA-antagonists for the treatment of Parkinson's disease have been suggested. Quisqualate receptors (=AMPA/Alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate) are present in the basal ganglia in higher densities than NMDA-subtypes. We therefore tested the effects of NBQX (6-nitro-7-sulfamoylbenzo[f]quinoxaline-2,3(1H,4H)-dione), a selective AMPA-antagonist, in two animal models of Parkinson's disease.

24 rats were injected with the neurotoxin 6-OHDA into the left substantia nigra, 8 months previously. NBQX administered alone (0.78, 3.13 or 12.5 mg/kg i.p.) did not induce ipsiversive or contraversive rotations (given as 36° turns in table 1). However, NBQX (12.5 mg/kg i.p.) in combination with a threshold dose of L-Dopa (25 mg/kg i.p.), after pretreatment with the peripheral decarboxylase inhibitor benserazide (100 mg/kg i.p.) significantly stimulated of contraversive rotations. Common marmosets were rendered parkinsonian by subcutaneous administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and treated with NBQX (6.25 mg/kg i.p.) and L-Dopa (20 mg/kg i.p.) plus benserazide (20 mg/kg i.p.). L-Dopa had no effect when given alone and NBQX reduced locomotor activity. Co-administration of both drugs ameliorated parkinsonian symptomatology, followed by a significant stimulation of well coordinated locomotor activity for 2 hours.

Table 1: Efects of NBQX plus L-Dopa on rotations (rats) and locomotor activity (monkeys)

	Mean counts per 120 min (SEM)				
	Veh+Veh	Veh+L-Dopa	NBQX+Veh	NBQX+L-Dopa	N
Rotations (rats)	2954 (498)**	6177 (1824)*	1247 (284)**	13421 (3059)	6
(Tukey test vs NBQX+L-Dopa, * p<0.05, ** p<0.01)					
Locomotion (monkeys)	1848 (385)	1858 (586)	965 (493)	4861 (523)	4
(Page test, ordered alternative: NBQX+Veh < Veh+Veh < Veh+L-Dopa < NBQX+L-Dopa, p<0.01)					

These data indicate the potential usefulness of AMPA-antagonists for symptomatic treatment of Parkinson's disease when combined with L-Dopa.

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74P CHARACTERISATION OF THE BINDING OF [^{3}H]L-689,560, AN ANTAGONIST FOR THE GLYCINE SITE ON THE NMDA RECEPTOR, TO RAT BRAIN MEMBRANES

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Recently, a series of trans-2-carboxy-4-substituted tetrahydroquinolines have been described (Leeson et al, 1991), which are potent antagonists of the glycine site on the N-methyl-D-aspartate (NMDA) receptor. 4-Trans-2-carboxy-5,7-dichloro-4-phenylaminocarbonylamino-1,2,3,4-tetrahydroquinoline (L-689,560), which inhibits [^{3}H]glycine binding with an IC_{50} of 7.8nM (Leeson et al, 1991), has subsequently been radiolabelled with tritium (28.4 Ci/mmol; Amersham, custom synthesis). We have evaluated the binding characteristics of [^{3}H]L-689,560 as a putative radioligand for the glycine site on the NMDA receptor in rat brain membranes.

P_2 membranes were prepared from rat cerebral cortex and hippocampus as described previously (Foster and Kemp, 1989). For [^{3}H]L-689,560 binding, 100 µg of membrane protein were incubated at 4°C for 45 or 120 min with 50mM Tris-acetate buffer (pH 7.0) and 0.1-1000nM [^{3}H]L-689,560 in a final volume of 0.5ml. Non-specific binding was determined with 1mM glycine and bound radioactivity was separated by filtration through Whatman GF/B filters.

[^{3}H]L-689,560 (1nM) showed a high degree of specific binding (95±0.6% of total, mean±SEM, n=4). Saturation analysis of [^{3}H]L-689,560 binding (n=3) gave a B_{max} of 4.15±0.9 pmoles/mg protein (arithmetic mean±SEM) and a K_D value of 2.97 (2.14,4.13)nM (geometric mean(-SEM,+SEM)). The B_{max} value is equivalent to that observed for [^{3}H]glycine in this membrane preparation. A number of glycine site ligands were assessed for their ability to displace [^{3}H]L-689,560 binding. The following IC_{50} values (µM; geometric mean(-SEM,+SEM);n=3) were obtained: glycine: 0.179(0.159,0.202); D-serine: 0.338(0.247,0.462); L-serine: 43.9(40.4,47.6); R(+)amino-1-hydroxypyrrolid-2-one ((+)HA-966): 9.67(7.82,12.0); S(-)HA-966: 654(494,866); 7-chlorokynurenic acid: 0.315(0.230,0.430); 5-iodo-7-chlorokynurenic acid: 0.014(0.011,0.017). A comparison of the IC_{50} values for 21 compounds (including agonists, antagonists and partial agonists) in the [^{3}H]glycine and [^{3}H]L-689,560 binding assays indicated a 1:1 correlation with a correlation coefficient of 0.97.

The characteristics of [^{3}H]L-689,560 binding are those expected for a selective interaction with the glycine site on the NMDA receptor. [^{3}H]L-689,560 is an improved high-affinity radioligand for this site, and will enable detailed studies of the interactions of glycine site antagonists with the NMDA receptor (see Gulati and Foster and Grimwood, Wilde and Foster, this meeting).

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75P THE ACTIONS OF 1S,3R-ACPD, A GLUTAMATE METABOTROPIC RECEPTOR AGONIST, IN AREA CA1 OF RAT HIPPOCAMPUS

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In addition to gating ionotropic receptors, excitatory amino acids such as L-glutamate activate the G-protein coupled metabotropic receptor causing an increase in phosphoinositide metabolism and release of intracellular calcium. A selective agonist for this receptor is the 1S,3R enantiomer of ACPD (1-aminocyclopentane-1,3-dicarboxylic acid) (Irving et al 1990). We have investigated the electrophysiological actions of 1S,3R-ACPD using a grease-gap method for the hippocampal slice as described by Blake et al (1988). Experiments were performed on slices obtained from female rats (5-8 weeks old) and perfused with a standard Mg^{2+}- containing perfusate at 28 °C. D.C potentials and field EPSPs were recorded across a grease barrier, separating the alveus from the isolated CA1 region, in response to the addition of agonists and electrical stimulation of the stratum radiatum, respectively.

1S,3R-ACPD (200-600 μM) elicited a slow, reversible and dose-dependent D.C. shift (n=11) in a direction opposite to the depolarising shifts caused by N-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA). In contrast 1R,3S-ACPD (100-1000 μM) was inactive (n=10). In a further 3 slices, 1R,3R-ACPD (10-50 μM) and 1S,3S-ACPD (100-500 μM) produced dose dependent depolarising responses which were selectively and reversibly blocked by the NMDA antagonist, D-2-amino-5-phosphonopentanoate (50 μM). In order to investigate whether the D.C. shift induced by 1S,3R-ACPD reflected a membrane hyperpolarisation, we altered the membrane potential of CA1 neurones by continuous perfusion of 1 or 2 μM AMPA. Responses to 1S,3R-ACPD during this tonic depolarisation were increased (n=4), as were depolarising responses to NMDA presumably by alleviation of the Mg^{2+} block of the NMDA receptor-channel.

In addition to causing a hyperpolarisation, 1S,3R-ACPD (200-600 μM) depressed the evoked field EPSP in agreement with microelectrode studies (Baskys and Malenka 1991).

Continuous perfusion of lower concentrations of 1S,3R-ACPD (10-100 μM) had no effect on the baseline D.C. potential or the evoked field EPSPs. However, depolarising responses to NMDA (25-40 μM) but not AMPA (5-15 μM), were potentiated (162-216%; n=4) in a readily reversible manner. Selective potentiation (164-194%; n=3) of responses to NMDA was also observed in a further 3 slices treated with 200 nM tetrodotoxin, ruling out the possibilty of a transsynaptic mechanism.

These data add to the diverse actions of metabotropic receptor activation in neurones. Perhaps most interesting is the potentiation of responses to NMDA which may have implications for synaptic plasticity and neuropathology.

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76P RELEASE OF ENDOGENOUS ADENOSINE AND RELATED PURINES FROM THE RAT HIPPOCAMPUS *IN VIVO*

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The present experiments employed in vivo microdialysis to determine whether activation of receptors for N-methyl-D-aspartate (NMDA) could induce purine release.

Male rats were used under urethane anaesthesia (1.25 g/kg, i.p.). Microdialysis probes, with 4 mm polyacrylonitrile membrane (HOSPAL), were lowered to the hippocampus (tip position AP: 5.6, L: 5.0, V: 8.0 mm; Paxinos & Watson, 1982). The probes were perfused continuously with artificial CSF (NaCl 125, $NaHCO_3$ 27, KCl 2.5, KH_2PO_4 0.5, $MgSO_4$ 1.2, $CaCl_2$ 1.2 Glucose 10 mM, pH 7.4) at 2 µl/min, and perfusate was collected every 20 min. The purine content of the dialysate was determined using HPLC with UV detection. The mobile phase was 0.01 M sodium dihydrophosphate with 6% methanol. After one hour's equilibration with ACSF, basal release was stable and sample collection was begun after a further hour.

NMDA, veratridine (Vtd), and K^+ (for 5 min) increased the release of purines (Table 1). As the concentration of NMDA increased, the duration of release was greater. Verapamil (100 µM) and Cd^{++} (300 µM) did not alter the basal or evoked release. A second stimulation by NMDA 200 µM or K^+ 100 mM also resulted in release which could be reduced or abolished by 2AP5.

Table 1 Purine release from the rat hippocampus (pmol/20 µl loop)

	Adenosine	Inosine	Hypoxanthine	Xanthine
Basal	2.2 ± 0.2 (24)	3.0 ± 0.2 (22)	8.7 ± 0.8 (18)	15.0 ± 1.3 (17)
NMDA (200 uM)	10.0 ± 2.4 (3)*	8.9 ± 1.6 (3)*	25.1 ± 0.5 (3)*	19.2 ± 4.3 (3)
NMDA (2 mM)	11.1 ± 1.2 (4)*	13.5 ± 1.2 (4)*	49.9 ± 4.8 (4)*	24.0 ± 1.2 (4)*
Vtd (100 uM)	23.9 ± 8.7 (2)*	39 ± 14.4 (2)*	-	-
K^+ (100 mM)	9.3 ± 2.3 (9)*	10.0 ± 1.3 (9)*	36.5 ± 7.9 (9)*	18.5 ± 3.2 (9)*

Values are mean ± s.e.m.(n). * P<0.001

Adenosine and its metabolites can be released by neuronal excitation in the rat hippocampus in vivo. The failure of verapamil and Cd^{++} to block release may suggest a calcium-independent releasable pool. 2AP5 reduced both the K^+ and NMDA-evoked release implying that NMDA receptors are involved.

Acknowledgement: Supported by the SHHD. We thank Professor C.A. Marsden for assistance.

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77P EFFECTS OF GENTAMICIN ON GLUCOSE TRANSPORT, TRANSEPITHELIAL RESISTANCES AND CELL VIABILITY IN ESTABLISHED RENAL CELL LINES

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Aminoglycosides including gentamicin which are used in the clinical management of gram-negative infections exhibit significant nephrotoxicity. Cultured renal cells provide useful models to investigate mechanisms of nephrotoxicity. We have previously reported that gentamicin impaired cell growth and protein synthesis in cultured primary rat renal proximal tubular cells (McGlynn & Ryan, 1990). In the present study, we have used the established renal cell lines LLC-PK_1 (with characteristics of the proximal tubule) and MDCK (with characteristics of the distal tubule) with biochemical, electrophysiological and flow cytometric techniques to investigate mechanisms of gentamicin nephrotoxicity.

Cells were cultured in DMEM supplemented with 10% foetal calf serum and 2 mM glutamine and grown in either 24-multiwell plates or Millicell-HA microporous inserts coated with collagen as appropriate. Glucose uptake was measured using [^{14}C]-alpha-methyl-glucose. Transepithelial resistance of cells grown on microporous filters was measured using the Millicell electrical resistance system. Transepithelial flux of [^{14}C]-mannitol was also measured. Cell viability was assessed using propidium iodide and fluorescein diacetate in a BD FAC Star plus flow cytometer.

Glucose uptake was found to be Na-dependent and phlorizin-sensitive in LLC-PK_1 cells but not in MDCK cells. Gentamicin in the concentration range 100 μM to 10 mM significantly inhibited glucose uptake in LLC-PK_1 cells but not in MDCK cells. At 40 mins, control uptake was 35 $\pm$ 1.2 nmoles/mg protein, Gentamicin at 1 mM reduced uptake to 15.2 $\pm$ 3.1 in LLC-PK_1 cells ($P<0.05$). At confluencey, both LLC-PK_1 and MDCK monolayers developed transepithelial resistances. Gentamicin at concentration of 1 mM for 48 h resulted in significant reductions from 145 $\pm$ 3 to 115 $\pm$ 3 in the case of LLC-PK_1 (ohms.cm^2; mean $\pm$ s.e. mean n=8, $p<0.005$) and from 252 $\pm$ 7 to 165 $\pm$ 10 in the case of MDCK cells. Gentamicin also significantly increased the transepithelial flux of [^{14}C]-mannitol in both LLC-PK_1 and MDCK cells. Flow cytometric analysis of cell viability revealed that gentamicin had no significant effects at concentrations up to 1 mM in both cell types.

These results demonstrate that gentamicin had significant effects on membrane properties including glucose transport and transepithelial resistance prior to any significant effects on cell viability.

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78P EFFECTS OF THE NON-PEPTIDE ANGIOTENSIN RECEPTOR ANTAGONIST, DuP 753, ON BASAL RENAL FUNCTION AND ON THE RENAL EFFECTS OF ANGIOTENSIN II IN THE ANAESTHETISED DOG

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The recently reported non-peptide, specific angiotensin receptor antagonist, DuP 753 (Wong et al., 1990) is under investigation as a potential treatment for hypertension. This study has examined the effects of DuP 753 on basal renal function, and on the renal effects of local intra-renal artery (i.r.a.) infusions of angiotensin II (Ang II) in pentobarbitone-anaesthetised dogs.

Mean aortic blood pressure (MBP) and left renal artery blood flow (RBF) were measured, and the left ureter cannulated for the collection of urine and determination of urine output (U_V). The following parameters were also measured or derived: glomerular filtration rate (GFR), renal vascular resistance (RVR), absolute ($U_{Na}V$) and fractional (FE_{Na}) sodium excretion, plasma renin activity (PRA), and plasma aldosterone concentration. After administration of DuP 753 (5mg/kg + 20μg/kg/min i.v.), the effects of the antagonist alone were examined over three, 15 min time periods. Ang II (3-300ng/kg/min i.r.a.) was then infused for 15 min at each dose. The effects of Ang II were compared with those observed in antagonist-free dogs.

Table 1: Effects of DuP 753 (5mg/kg + 20μg/kg/min i.v.) on renal function in the anaesthetised dog.

	MBP (mmHg)	RVR (TPRU)	RBF (ml/min)	U_V (ml/min)	$U_{Na}V$ (μmol/min)	GFR (ml/min)	FE_{Na} (%)
Basal	111±7	11±1	85±5	0.35±0.05	83±9	25±2	2.2±0.2
+15 min	-18±4*	-25±3*	+10±2*	-29±13*	-35±14*	-32±9*	-6±16
+30 min	-14±3*	-13±4*	0±7	-28± 9*	-30±14*	-9±12	-26±9*
+45 min	-14±2*	-13±4*	0±6	-10± 7	-10±10	-17±12	+9±7

Values are mean percentage change ± sem (n=4) from basal measurements. * $P<0.05$ vs saline controls (n=4).

Following administration of DuP 753 (Table 1), sustained falls in MBP and RVR, and an initial increase in RBF were observed. GFR, U_V, and $U_{Na}V$ were reduced 15-30 min post-DuP 753, but these decreases were not sustained. 30 min post-dose, despite increasing PRA from 4.4±0.4 to 10.8±2.1ngAI/ml/h, DuP 753 caused a reduction in plasma aldosterone levels from 140±29 to 74±22pg/ml. In the absence of DuP 753, Ang II (1-100ng/kg/min i.r.a., n=5) had no effect on MBP, but caused a dose-related increase in RVR (14-117%), and decrease in RBF (12-51%), U_V (23-56%), $U_{Na}V$ (20-67%), FE_{Na} (18-49%), and GFR (10-37%). In the presence of DuP 753, Ang II dose-response curves on these renal parameters were displaced 5-20 fold to the right of those obtained in antagonist-free dogs.

These results indicate blockade by DuP 753 of the renal and systemic vasoconstrictor and steroidogenic effects of endogenous angiotensin. Additionally, this study suggests that DuP 753 is an effective antagonist of the renal haemodynamic and tubular effects of exogenous Ang II.

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79P THE ACTIVITY OF A CRUDE EXTRACT OF BOVINE ADRENAL MEDULLA AT α-ADRENOCEPTORS AND A NON-ADRENOCEPTOR, IMIDAZOLINE BINDING SITE CAN BE ACCOUNTED FOR BY ADRENALINE

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In a comparative study of several organs from the rat, Hensley *et al.*, (1989) reported that the highest concentration of the putative endogenous ligand for 'imidazoline' binding sites, clonidine-displacing substance (CDS), was found in the adrenal gland. However, since the extraction procedure adopted for CDS has a number of similarities to that earlier employed to extract adrenaline (ADR) (see: Aldrich, 1901), we have compared the activity of this catecholamine with a crude extract of the bovine adrenal medulla at α_1-, α_2-adrenoceptor and non-adrenoceptor, imidazoline binding sites.

The method for preparing CDS is essentially that previously described by Hensley *et al.*, (1989). α_1-, α_2-adrenoceptor binding sites in bovine cerebral cortex membranes were labelled with 0.5nM [3H]-prazosin and 0.5nM [3H]-clonidine, respectively. Non-specific binding was determined in the presence of 100μM noradrenaline (NA). Non-adrenoceptor, imidazoline binding sites in the rat kidney were labelled with 1nM [3H]-idazoxan as described by (MacKinnon *et al.*, 1991). The catecholamine content in a volume of the extract (able to produce 50% displacement of [3H]-clonidine binding) was determined by alumina adsorption followed by HPLC with electrochemical detection.

ADR and the bovine adrenal medulla extract produced concentration-dependent inhibition of the radioligands at α_1-, α_2- and non-adrenoceptor, imidazoline binding sites (Table 1). 0.1nl of the adrenal extract contained 6.4±0.5pmoles ADR and 2.2±0.5pmoles NA (n=3 batches). Based upon the these values, the estimated concentration of ADR in the volume of the assay medium containing the extract (Table 1) can account for the activity observed at each binding site

Table 1: pIC_{50} for ADR and the bovine adrenal medulla extract at various sites (n= mean of 3 separate batches).

	[3H-CLON]	[3H]-PRAZ	[3H]-IDAZ
-log extract vol (ml) in 1ml assay	6.98±0.03	4.75±0.08	3.12±0.3
-log [ADR] (M) in extract assay	8.2	5.96	4.32
-log [ADR] (M)	7.92±0.13	5.76±0.01	4.53±0.2

In conclusion, we have found no evidence to support the view that the adrenal gland contains the putative endogenous ligand, CDS, for imidazoline receptors (Hensley, 1989). Rather, it appears that the activity of adrenal medulla extract at α_1-, α_2- and non-adrenoceptor, imidazoline binding sites can be entirely accounted for by the endogenous catecholamine ADR.

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80P A CRUDE EXTRACT OF BOVINE LUNG DISPLACES LIGANDS FROM α_2-ADRENOCEPTORS AND A NON-ADRENOCEPTOR, IMIDAZOLINE BINDING SITE

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In a comparative study of several organs from the rat, Hensley *et al.*, (1989) reported that the lowest concentration of the putative endogenous ligand for 'imidazoline' binding sites, clonidine-displacing substance (CDS; Atlas and Burnstein , 1984), was found in the lung. In the present study we have prepared a crude extract of bovine lung and examined its ability to displace a radioligand at an α_1-, an α_2-adrenoceptor and a non-adrenoceptor, imidazoline binding site.

The method for preparing the crude extract of the bovine lung is essentially that previously described by Hensley *et al.*, (1989). α_1-, α_2-adrenoceptor binding sites in bovine cerebral cortex membranes were labelled with 0.5nM [3H]-prazosin and 0.5nM [3H]-clonidine, respectively. Non-specific binding was determined in the presence of 100μM noradrenaline (NA). Non-adrenoceptor, imidazoline binding sites in the rat kidney were labelled with 1nM [3H]-idazoxan as described by (MacKinnon *et al.*, 1991). The catecholamine and cation content of the extract were also determined.

The crude lung extract produced a concentration-dependent inhibition of specific [3H]-clonidine and [3H]-idazoxan binding, the maximum volume employed, 60μl/ml incubation, caused 74.6±2.3% (n=4) and 100% inhibition (n=3), respectively. No activity at α_1-adrenoceptors was detected. The extract was 5-fold more potent against at the imidazoline binding site compared to the α_2-adrenoceptor binding site (Table 1). The observed activity cannot be accounted for by either adrenaline or noradrenaline (the IC_{50} volume of the extract for α_2-adrenoceptors contained less than 1pmole of either catecholamine) nor by monovalent cations (Table 1) In a separate series of experiments, the IC_{50} for Na^+ at the α_2-adrenoceptors binding site was 40mM (n=5), and for K^+ at the non-adrenoceptor, imidazoline binding site was 24mM (n=2).

Table 1: Properties of the crude bovine lung extract at various ligand binding sites (n= mean of 3-4 separate batches)

	[3H]-CLON	[3H]-PRAZ	[3H]IDAZ
-log extract vol (ml) in 1ml assay	1.65±0.05	>1.22	2.38±0.15
[cation] in extract assay vol	3mM (Na^+)		0.5mM (K^+)
-log [NA] (M) in extract assay vol	<9.0		<9.7

In conclusion, we have shown that a crude extract of the bovine lung contains a non-catecholamine substance(s) which displays a 5-fold greater potency at non-adrenoceptor, imidazoline binding sites compared with α_2-adrenoceptor binding sites. These preliminary finding suggest that this substance may be identical or related to 'CDS', previously extracted from both central and perpiheral tissues (Atlas and Burnstein, 1984; Hensley *et al.*, 1989) .

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81P EFFECTS OF ADENOSINE ON RAT CAROTID BODY CHEMORECEPTORS

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Adenosine increases ventilation when administered in man (e.g. Watt et al, 1987) and this effect has been attributed to activation of carotid body chemoreceptors, mainly on the basis that adenosine excites cat carotid chemoreceptors (McQueen & Ribeiro, 1981) and cutting the carotid sinus nerves in anaesthetized rats abolishes adenosine-induced hyperventilation (Monteiro & Ribeiro, 1987). The present study was undertaken to examine the effects of adenosine on both ventilation and carotid chemoreceptor discharge in anaesthetized rats so as to establish the extent to which activation of carotid chemoreceptors contributes to adenosine-induced hyperventilation.

Experiments were performed on male Wistar rats anaesthetized with pentobarbitone (60 mg/kg i.p., then i.v. supplements) in which respiration was measured by an electrospirometer, and chemoreceptor discharge was recorded from a carotid sinus nerve in spontaneously breathing animals (see McQueen & Ribeiro,1981). Drugs were injected i.v. via a femoral vein, or into a common carotid artery (i.a.) through a cannulated external carotid artery.

Adenosine (4-1873 nmole i.v. or i.a.) caused a variable delayed increase in ventilation (maximum about 50%) which tended to be dose-related. After denervating the carotid body chemoreceptors by cutting the carotid sinus nerves (n=9 rats) there was a slight attenuation of the ventilatory increase following adenosine (i.v. or i.a.), but the reductions were not statistically significant. Neural recordings in five rats showed adenosine had very little immediate effect on chemoreceptor discharge, but caused a delayed increase in frequency in some experiments, which coincided with a fall in systemic B.P..

The results suggest that adenosine does not cause significant excitation of carotid body arterial chemoreceptors, and thus make it unlikely that these sensors play a major role in adenosine-induced hyperventilation in the rat.

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82P PRODUCTION AND CHARACTERISATION OF ANTISERA DIRECTED AGAINST THE $G_{11\alpha}$ GENE PRODUCT

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G11 and Gq are two highly homologous heterotrimeric guanine nucleotide binding proteins. cDNA's corresponding to the α subunits of these G-proteins have recently been isolated (Strathmann and Simon, 1990). These G-proteins have been implicated as regulators of phospholipase C β1 (Taylor *et al.*, 1991) Smrcka *et al.*, 1991). Using the primary amino acid sequence predicted for these G-proteins we have generated an antiserum against a conjugate of keyhole limpet haemocyanin and a synthetic peptide corresponding to the carboxyl-terminal decapeptide common to G11 and Gq. This antiserum (CQ2) identifies an apparently single polypeptide of 41 kDa in all tissues and cell lines which we have examined but shows no cross-reactivity with synthetic peptides representing the carboxyl-terminal regions of G_i1, G_i2, G_i3, G_o, G_z, G12 or G13. Immunoblotting of membranes of pertussis toxin pretreated cells with an antiserum which identifies $G_i2\alpha$ indicated that the addition of ADP-ribose to a G-protein hinders its mobility through SDS-PAGE. No alteration in mobility of the polypeptide identified by CQ2 was produced by such treatment indicating that, as expected, G11 is not a substrate for this modification. Cholera toxin treatment of cells produces a marked down regulation of levels of $G_s\alpha$. Such treatment also had no effect on the levels of the polypeptide identified by antiserum CQ2.

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83P SYNERGISTIC INTERACTION BETWEEN BRADYKININ AND INTERLEUKIN-1 IN ANGIOGENESIS

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There is accumulating evidence from *in vitro* studies that some neuropeptides and autacoids can also act as growth factors (Woll & Rozengurt, 1989). Using an *in vivo* model, we recently showed that substance P, calcitonin gene related peptide and vasoactive intestinal peptide induced neovascularisation (Fan & Hu, 1991). Here we report that bradykinin (BK) and interleukin-1 (IL-1) acted in synergy to promote angiogenesis in the same model. In addition, we also tested the angiogenic potential of angiotensin II (AII).

Sterile polyether sponges with attached cannulae were implanted subcutaneously in male Wistar rats (180-200g) and neovascularisation was assessed as a function of blood flow through the implants every two days over a period of 14 days, using a ^{133}Xe clearance technique (Andrade et al., 1987). To exclude the possible acute effects of the test substances on the microvasculature, they were always injected via the cannulae into the sponges 24 h before ^{133}Xe measurements. Further control studies using laser Doppler flowmetry established that intradermal injection of these peptides, either alone or in combination, produced no significant changes of flux in normal rat skin at 0-20 min, 6 h,12 h and 24 h after injection.

Sponge implants receiving daily doses of 1000 pmol of BK or 3 pmol IL-1α showed significantly higher clearance values than the control group, e.g. the 6 min ^{133}Xe clearance value of the control group on Day 8 was 30.8±1.3% (n=8) while BK or IL-1α increased it to 42.6±1.5% and 42.5±2.3%, respectively (n=8, $P<0.01$). Histological studies of sponge sections stained with haematoxylin & eosin or a specific endothelial cell marker *Bandeirea simplicifolia* lectin I, isolectin B_4 showed increased cellularity and vascularity in the test sponges as compared to controls. Although lower doses (1 and 10 pmol) of BK or IL-1α (0.3 pmol) did not induce significant angiogenic responses, subthreshold doses of BK (10 pmol) and IL-1α (0.3 pmol) acted synergistically to produce a similar enhancement of angiogenesis (41.8±2.0% ^{133}Xe clearance on Day 8, n=12, $P<0.01$). Furthermore, this effect of BK was totally abolished by selective BK receptor antagonist des-Arg^9, [Leu^8]-BK, but not by Ac-D-Arg-[Hyp^3, D-Phe^7, Leu^8]-BK, suggesting that the activation of B_1 receptors probably mediates the angiogenic response of the tissue to exogenous BK. Parallel studies showed that daily administration of 1000 pmol AII caused an intense neovascularisation as measured by ^{133}Xe clearance and histologically, while 10 or 100 pmol AII were ineffective. The effect of AII was abolished by selective AII receptor antagonist saralasin, and there was no angiogenic synergy between AII (10 pmol) and IL-1α (0.3 pmol).

Thus, BK and AII could be involved in the modulation of angiogenesis. Furthermore, the synergistic interaction between BK and IL-1α may contribute to the excessive neovascularisation in chronic inflammatory diseases such as rheumatoid arthritis.

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84P INHIBITION OF THROMBIN-INDUCED INCREASES IN ENDOTHELIAL PERMEABILITY BY FORSKOLIN AND ATRIOPEPTIN II

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Factors regulating inflammatory oedema are poorly understood. It is known, however, that thrombin increases the transfer of macromolecular solutes across bovine pulmonary arterial endothelial (BPAEC) monolayers by a mechanism that is inhibited by elevation of cyclic AMP content (Minnear et al., 1989). We wished to determine if elevation of cyclic GMP content could similarly inhibit trans-endothelial transfer, and establish if blockade of calcium mobilisation was involved.

Endothelial barrier function was assessed by measuring the transfer of trypan blue-labelled albumin (4%) across monolayers of BPAEC grown on Transwell membranes (Gudgeon & Martin, 1989). Intracellular calcium $[Ca^{2+}]_i$ was measured in BPAEC using fura-2 (Buchan & Martin, 1991).

Unstimulated equilibration of albumin across BPAEC was 7.4±0.5% (n=30), and this was increased to 13.8±1.1% (n=18) by thrombin ($1Uml^{-1}$) and to 16.6±1.3% (n=12) by phorbol myristate acetate (PMA, 0.6μM). Forskolin (30μM), an activator of adenylate cyclase, had no effect on unstimulated equilibration of albumin, but abolished the increases stimulated by thrombin or PMA. Atriopeptin II (100nM), an activator of particulate guanylate cyclase, had no effect on unstimulated equilibration of albumin or that stimulated by PMA, but abolished that stimulated by thrombin. Resting $[Ca^{2+}]_i$ in BPAEC was 96±5nM (n=35). Thrombin ($1Uml^{-1}$) increased $[Ca^{2+}]_i$ to a peak of 280±34nM (n=13), which decayed to a sustained plateau of 162±13nM after 5min. Forskolin (30μM; 5min) had no effect on resting $[Ca^{2+}]_i$ or on the thrombin-induced peak increase in $[Ca^{2+}]_i$. It did, however, increase $[Ca^{2+}]_i$ by 67±7nM (n=6) when added during the plateau phase of the increase stimulated by thrombin. In contrast, atriopeptin II (100nM; 5min) had no effect on resting $[Ca^{2+}]_i$ or on the magnitude of the peak and plateau increases in $[Ca^{2+}]_i$ stimulated by thrombin. PMA (0.1μM; 5min) inhibited both the peak and plateau increases in $[Ca^{2+}]_i$ stimulated by thrombin.

In conclusion, elevation of endothelial $[Ca^{2+}]_i$ appears not to be a prerequisite for stimulation of macromolecular transfer. Activation of protein kinase C increases macromolecular transfer and this is inhibited by elevation of cyclic AMP, but not cyclic GMP content. Cyclic GMP may inhibit the thrombin-induced increase in macromolecular transfer by an action on the stimulus-coupling mechanism proximal to stimulation of protein kinase C.

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85P IDENTIFICATION AND PARTIAL CHARACTERISATION OF A RECEPTOR FOR PLATELET ACTIVATING FACTOR ON GUINEA-PIG PERITONEAL MACROPHAGES

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It is now recognised that the alveolar macrophage may be an important effector cell in asthma pathogenesis. Previous studies have demonstrated that platelet activating factor (PAF), a potent lipid mediator implicated in asthma, activates macrophages (Lambrecht & Parnham, 1986; Stewart & Dusting, 1988). In this communication we report the identification and characterisation of a macrophage PAF receptor utilising the radiolabelled PAF antagonist [^{3}H] WEB 2086.

Macrophages were harvested from the peritoneal cavity of human serum-treated male guinea-pigs and purified on discontinuous Percoll gradients. Cells (purity > 94%) were resuspended at the required concentration in HEPES-buffered physiological salt solution and used immediately for binding studies as described previously (Ukena *et al.*, 1989).

Table I. Inhibition of [^{3}H] WEB 2086 Binding to Intact Macrophages by Selected Drugs

Drug	K_I (nM)	Slope(n_H)
BN 50739	01.9 ± 0.2	-1.3 ± 0.1
UK 74505	02.6 ± 0.2	-1.1 ± 0.1
STY 2108	04.3 ± 0.4	-1.1 ± 0.1
BN 50741	04.7 ± 0.9	-1.2 ± 0.2
WEB 2086	06.4 ± 1.2	-1.2 ± 0.2
WEB 2170	10.5 ± 0.2	-1.1 ± 0.1
C_{16}-PAF	15.3 ± 8.0	-0.4 ± 0.01
Lyso-PAF	> 10000	-

[^{3}H] WEB 2086 bound to intact guinea-pig macrophages in a concentration-dependent manner. This binding was specific (>90% at the Kd), saturable, reversible and proportional to cell density (up to 8 x 10^6 cells/sample). Scatchard anaylsis of these data revealed that [^{3}H] WEB 2086 identified a homogeneous population of non-interacting high affinity (Kd: 6.6 ± 0.7 nM at 25°C, n = 6) binding sites with a Bmax of 33,600 ± 3600 (n = 6) sites per cell. Kinetic studies showed that the association and dissociation of [^{3}H] WEB 2086 was slow with respective $t_{1/2}$ values of 14.6 ± 0.8 (n = 3) and 49.6 ± 6.6 (n = 3) min respectively. Similarly, the displacement of [^{3}H] WEB 2086 (10 nM) by PAF (5 µM) was slow ($t_{1/2}$ = 40.7 ± 4.5 min; n = 4). The kinetically determined Kd calculated from the association (K_{ON}: 2.9 x 10^6 M^{-1}. min^{-1}) and dissociation (K_{OFF}: 1.4 x 10^{-2}. min^{-1}) rate constants (i.e. K_{OFF}/K_{ON}) was 5 nM. The ability of a number of drugs to compete with [^{3}H] WEB 2086 for binding to the macrophage was determined (Table I). With the exception of C_{16}-PAF (which yielded a shallow competition curve) and *lyso*-PAF (which was inactive), all other drugs inhibited [^{3}H] WEB 2086 binding with slopes not significantly different from unity.

It is concluded that guinea-pig peritoneal macrophages express a receptor(s) for PAF for which WEB 2086 has high affinity. The shallow inhibition curve obtained with PAF suggests that the PAF receptor on this cell type is either heterogeneous or exists in different affinity states. The slow rate at which [^{3}H] WEB 2086 dissociates from this receptor may explain why this antagonist behaves in an apparently non-competitive manner in some cell types (e.g. Giembycz *et al.*, 1990).

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86P PLATELET ACTIVATING FACTOR STIMULATES A PERTUSSIS TOXIN-SENSITIVE GTPASE ACTIVITY IN GUINEA-PIG EOSINOPHIL MEMBRANES

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Platelet activating factor (PAF) is an ether-linked phospholipid that activates several cells involved in inflammatory reactions, including neutrophils, basophils, macrophages and eosinophils. In human platelet and neutrophil membranes, PAF causes stimulation of a GTPase activity that represents the activated (i.e. GTP-liganded) α-subunit of a guanine nucleotide-binding regulatory protein (G-protein). The characteristics of the GTPase-stimulation differ between the cell types and this may indicate the existence of multiple classes of PAF receptors or their coupling to different G-proteins (Hwang *et al.*, 1988). In this study we have sought to identify a PAF-stimulated GTPase in cell membrane preparations from guinea pig eosinophils and to characterize the enzyme in terms of its sensitivity to sodium ions and to cholera (CTx) and pertussis toxins (PTx).

Eosinophils were isolated from peritoneal lavages of human serum-treated (1 ml/week) male Dunkin-Hartley guinea pigs by differential centrifugation over discontinuous Percoll density gradients. Cells were lysed in hypoosmotic buffer (Tris-HCl 10 mM, $MgCl_2$ 5 mM, EDTA 2 mM, (±)DTT 2 mM, PMSF 1 mM; pH 7.0) by 5 cycles of freeze-thawing and sonication. Particulate fractions of cell lysates were washed twice, resuspended in lysis buffer supplemented with leupeptin 1 µM, pepstatin A 1 µM and soybean trypsin inhibitor 5 µg/ml and stored at -70°C. GTPase was assayed as described (Hwang, 1988).

PAF caused a concentration-dependent stimulation of eosinophil membrane GTPase, up to a maximum of 112% of control at 10 µM, with an EC_{50} of 74.6 ± 42.9 nM (n = 5). This stimulation was inhibited by the specific PAF receptor antagonist, WEB 2086, with an IC_{50} of 207 ± 124 nM *vs.* 1 µM PAF (n = 3). Stimulation of GTPase by 1 µM PAF was enhanced in the presence of 150 mM NaCl (6.8 ± 2.0% above control (n = 3, $p < 0.05$) *vs.* 2.4 ± 1.4% above control in the absence of Na^+ (n = 3, N.S.)) and was abolished by PTx (50 µg/ml) but not by CTx (100 µg/ml). These characteristics are different from those displayed by the PAF receptor-associated GTPase in human neutrophils or platelets. We conclude that PAF receptors on guinea pig eosinophils are coupled via a PTx-sensitive G-protein which does not have an absolute requirement for Na^+ and is distinct from the G-proteins associated with PAF receptors in human neutrophils and platelets.

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87P PYOCYANIN OR METHYLENE BLUE POTENTIATES THE ANTIAGGREGATORY ACTION OF SODIUM NITROPRUSSIDE BY A MECHANISM INVOLVING THE RELEASE OF NITRIC OXIDE

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Pyocyanin (PYO, 1-hydroxy-5-methyl-phenazinum betaine) is a redox pigment produced by P. aeruginosa which inhibits the biological activity of EDRF, probably by scavenging nitric oxide (NO) (Warren et al, 1990). We have previously shown (Zembowicz et al, 1991) that PYO and another structurally related redox dye, methylene blue (MeB) potentiate relaxations of rabbit aortic strips induced by sodium nitroprusside (NaNP), but inhibit those induced by other NO-donors such as glyceryl trinitrate, a metabolite of molsidomine (SIN-1) or S-nitroso-N-acetylpenicillamine (SNAP). Here we show that PYO and MeB potentiate the antiaggregatory action of NaNP in human or rabbit platelet rich plasma (PRP) whereas they inhibit that of SNAP. Human or rabbit PRP was preincubated for 3 min (37°C, 1000 rpm) in a Payton dual channel aggregometer. Collagen at concentrations (0.5-1.5μg/ml) inducing 80% of maximal aggregation was added to the platelet suspension and the change in light transmission was measured for a further 4 min. PYO (0.01-10μM) or MeB (0.01-10μM) was added to the platelet suspension 2 min before collagen. NaNP or SNAP was added 1 min before collagen. Oxyhaemoglobin (oxy-Hb,10μM) was added to PRP at different time periods (30sec-5min) after stimulation with collagen.

PYO or MeB alone (at concentrations up to 10μM) had no effect on platelet aggregation. PYO or MeB (0.3-10μM) potentiated the antiaggregatory action of the threshold inhibitory concentrations of NaNP (6.5±1μM,) which alone produced a 7.5±1% inhibition of platelet aggregation (n=4, $p<0.0001$ one-way ANOVA). The combination of this concentration of NaNP with PYO (3μM) or MeB (3μM) completely abolished platelet aggregation (95±4%, 97±2% respectively, Bonferroni $p<0.001$). This inhibition was reversed in a time-dependant manner by administration of oxy-Hb (10μM) added 30 sec to 5 min after stimulation with collagen (n=4). Thus, when added in the presence of PYO or MeB after 30 sec, oxy-Hb caused 91±4% or 75.5±2% ($p<0.001$, one sample T test) reversal of inhibition, and after 2 min, 33±3% or 47±3% ($p<0.002$, one sample T test) respectively. No reversal was observed after 5 min. In contrast, PYO or MeB (10μM) inhibited the antiaggregatory action of SNAP (20 μM, Bonferroni $p<0.05$, n=3). The time-dependent reversal of platelet aggregation by oxy-Hb added after a proaggregatory stimulus is selective for NO or NO-donors but not for iloprost, a prostacyclin analogue, or phosphodiesterase inhibitors (Salvemini et al, 1990). Thus, we conclude that the potentiation of the antiaggregatory action of NaNP by PYO or MeB involves facilitation of the release of NO from the molecule of NaNP most probably due to the redox properties of these dyes.

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88P EFFECT OF Ro 31-8220, A NOVEL PKC INHIBITOR, ON HUMAN PLATELET PHOSPHORYLATION AND SECRETION

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Human platelet activation by thrombin is associated with production of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DG). IP3 releases Ca^{2+} from the dense tubular system and this, in turn, activates myosin light chain kinase (MLCK); diacylglycerol activates protein kinase C (PKC). Both MLCK and PKC phosphorylate myosin light chain (20 kDa) but at distinct sites; the major substrate for PKC in platelets is pleckstrin (47 kDa) which has no known function. The aim of this study is to establish further the role of PKC in secretion of 5-hydroxytryptamine (5-HT) using the PKC inhibitor Ro 31-8220.

Stimulation of platelets with thrombin (1 unit/ml) for 30s increased phosphorylation of pleckstrin and myosin light chain by ~400% and ~220% respectively. At a maximally effective concentration (3μM), Ro 31-8220 reduced thrombin-induced phosphorylation of myosin light chain and pleckstrin by 20% and to below basal levels respectively. In contrast, the non-selective inhibitor, staurosporine (1μM) completely inhibited phosphorylation of both proteins. Maximal activation of PKC by PDBu (300nM) increased phosphorylation of pleckstrin by 419% ± 6% and myosin light chain by 62% ± 5%; Ro 31-8220 (3μM) reduced these to below basal levels in both cases. Myosin light chain phosphorylation induced by A23187 (1μM) was inhibited by Ro 31-8220 from 70% ± 11% to 30% ± 8% above basal, while pleckstrin phosphorylation (206% ± 27%) was inhibited completely.

Ro 31-8220 inhibited thrombin-induced 5-HT secretion; at a maximally effective concentration (3μM), secretion was reduced from 57% ± 2.8% to 6.3% ± 1.6%. A23187 (1μM)-induced secretion was potentiated by PDBu from 17% ± 0.6% to 48% ± 4.8%, on its own PDBu induced no significant secretion. Ro 31-8220 inhibited secretion to A23187 in the absence and presence of PDBu to 7.1% ± 1.6% and 10.4% ± 2.5% respectively.

The present results are consistent with Ro 31-8220 acting as a selective PKC inhibitor. Ro 31-8220 inhibited completely phosphorylation of pleckstrin by thrombin, A23187 and PDBu; myosin light chain phosphorylation by PDBu was inhibited completely, but that induced by thrombin and A23187 was inhibited partially. As myosin light chain is a substrate for both MLCK and PKC, we suggest that Ro 31-8220 is inhibiting only the PKC-induced phosphorylation. Inhibition of PKC by Ro 31-8220 reduced secretion by thrombin and A23187 in the presence of PDBu to less than 10%. These results suggest that secretion is a Ca^{2+}-driven pathway which is markedly potentiated by activation of PKC.

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89P INVESTIGATION OF THE EFFICACY OF A PANEL OF ANTI-HUMAN TNF ANTIBODIES IN THE CONSCIOUS RABBIT MODEL

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Tumour necrosis factor (TNF) is an endogenous polypeptide which, when present in high concentrations, has been implicated in the pathogenesis of endotoxic shock (Tracey et al, 1987). Much interest has been shown in the therapeutic use of anti-TNF monoclonal antibodies (Mabs), however, the species specificity of such antibodies has prevented the study of efficacy against human TNF in models other than primates or man. The present study investigated the efficacy of a panel of murine anti-human TNF Mabs in conscious rabbits given recombinant human TNF (rec huTNF).

Male New Zealand White rabbits (2-2.4kg) were anaesthetised with sodium pentobarbitone (40mg/kg iv) and the left femoral artery and vein cannulated for measurement of blood pressure and drug administration, respectively. Experiments were carried out the following day, whilst rabbits were fully conscious. Internal body temperature was monitored by a rectal thermistor probe and recorded by an Isothermix recording device. After a 60 minute equilibration period either saline or antibody was administered by a single iv bolus and 15 minutes later either saline or rec huTNF, (1ug/kg), was given. Blood samples were taken every 60 minutes for a further 300 minutes and the plasma analysed for rec huTNF levels by both ELISA and the L929 bioassay.

The three murine anti-TNF Mabs assessed were given in differing doses: CB0006 (0.01-10mg/kg); CB0010 or CB0011 (0.001-0.1mg/kg). Each rabbit received one treatment only. The plasma TNF levels were compared using 95% confidence intervals whilst the maximum changes in temperature in the first 120 minutes were plotted against dose of antibody and relative potencies estimated using parallel-line assay techniques.

Plasma TNF levels at 60 min in the rec huTNF alone groups were 971 ± 165 (mean ± 95% CI) and 740 ± 250 pg/ml measured by the ELISA or bioassay, respectively, which declined over 5 hours showing a half-life of 60 minutes. This dose of rec huTNF caused transient pyrexia, peaking at 0.5-0.9 °C, between 45 and 75 minutes post administration. Furthermore, administration of rec huTNF did not elicit the release of endogenous rabbit TNF. Pretreatment with anti-TNF antibodies showed dose-dependent inhibitions of the TNF-induced pyrexia. Total inhibition of the temperature rise was seen at 1 mg/kg CB0006 whilst both CB0010 and CB0011 were similarly effective at 0.1mg/kg. Both plasma TNF assays showed dose-dependent reductions of TNF-like activity in the presence of each antibody. The rank order of potency for neutralising plasma TNF being similar to that for their anti-pyretic effect (CB0011≥CB0010 > CB0006). However, the relative potencies of CB0010 and CB0011 were at least 100 fold greater than CB0006 in their ability to remove circulating TNF. This difference was less pronounced in their inhibition of the TNF-induced pyrexia as CB0010 and CB0011 were approximately 5.5 and 7-fold ($p < 0.005$), respectively, more potent than CB0006.

The conscious rabbit model gives both a biological readout (pyrexia) and a measure of TNF neutralising ability, showing that an inappropriate species can be used to assess the relative potencies of anti-human TNF antibodies despite their strict species specificity.

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90P PGE_2 POTENTIATES PLASMA EXUDATION IN RABBIT SKIN BY TWO DIFFERENT MECHANISMS

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The aim of this study was to test the hypothesis that dilatation is the primary mechanism by which PGE_2 potentiates plasma exudation in rabbit skin (Wedmore & Williams, 1981). The ability of a number of PGE analogues, showing differential selectivity at the 3 subtypes of the PGE receptor, was tested, firstly to potentiate plasma exudation induced by bradykinin (BK, 0.5 µg i.d.) and formyl-methionyl-leucyl-phenyl-alanine (FMLP, 20 ng i.d.), measured by leakage of ^{125}I-albumin; and secondly to induce dilatation in rabbit skin, measured by ^{133}Xe clearance.

Table 1 Maximal potentiation of plasma exudation and dilatation in rabbit skin, and relaxation of guinea-pig trachea in vitro.

	max resp.(%BK)	max resp.(%FMLP)	{max%Δ blood flow	IC_{50}(ng)}	trachea IC_{50}(ng/ml)
EP_2/EP_3 agonists					
PGE_2 (1 µg) ✣	211.7 ± 27.4	151.7 ± 19.9	119.2 ± 26.0	31.6	75.7 ± 11.5
misoprostol (1 µg)	188.5 ± 26.2	141.0 ± 24.4	140.8 ± 27.3	25.1	15.9 ± 4.7
16,16-diMe PGE_2 (1 µg) *✣	120.5 ± 8.5	129.5 ± 38.7	159.8 ± 29.7	4.0	149.6 ± 41.1
enisoprost (1 µg) *	120.7 ± 13.6	136.8 ± 23.5	99.63 ± 20.4	10.0	66.0 ± 21.5
EP_2 agonists					
11-deoxy PGE_1 (2.5 µg)	96.2 ± 12.0	104.2 ± 30.1	108.1 ± 26.1	316.2	154.9 ± 45.1
butaprost (1 µg)	85.3 ± 17.2	86.5 ± 15.7	66.1 ± 14.1	≥1000.0	25.5 ± 2.7
mexiprostil (1 µg) ✣	78.5 ± 14.1	61.2 ± 21.1	51.2 ± 6.3	≥1000.0	>5000.0
11-deoxy PGE_2 -1 OH (5 µg) *	38.0 ± 6.4	10.1 ± 3.5	-6.35 ±8.2	>5000.0	174.0 ± 96.3
EP_3 agonists					
M&B 28,767 (1 ng) *	80.3 ± 11.3	16.6 ± 12.4	-9.8 ± 1.2	>1000.0	
sulprostone (10 ng) ✣	54.2 ± 13.1	14.2 ± 9.0	19.9 ± 18.0	>1000.0	
GR 63799X (10 ng) ✣	49.0 ± 10.4	23.8 ± 6.7	-29.9 ± 2.98	>1000.0	(mean ± s.e.m. n ≥4)

* analogues with TP activity were tested in rabbits given 2 mg/Kg i.v. of the TP antagonist GR 32191.
✣ analogues with EP_1 activity. Both TP and EP_1 activities are likely to oppose dilatation.

The simplest model is with a leukocyte-dependent mediator, which unlike BK, does not induce dilatation; the ability of the EP_2 agonists to potentiate FMLP correlates well with dilatation, and EP_3-selective agonists are inactive at both. In contrast, EP_3 analogues potentiate BK-induced exudation to a similar extent as some EP_2 analogues, but at lower concentrations. We have suggested before that BK-induced dilatation is potentiating an EP_3-selective leukocyte-dependent mechanism (Armstrong & Jones, 1991), and initial studies suggest that EP_3 agonists are chemoattractants for human neutrophils in vitro. Analogues with both EP_2/EP_3 activity potentiate BK to the greatest extent. These results suggest two distinct actions of PGE_2 : an EP_3 -leukocyte-dependent mechanism at low concentrations, and dilatation at higher concentrations. However, the receptor mediating dilatation in the skin varies from the conventional EP_2 receptor, i.e. relaxation of the guinea pig trachea after contraction by 17-phenyl PGE_2 (Table 1), and is more similar to that described for the rabbit jugular vein (Lawrence et al, 1989).

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91P EFFECTS OF THE NEW ANTIHISTAMINE SL 85.0324 ON HISTAMINE-INDUCED EICOSANOID RELEASE FROM THE ISOLATED PERFUSED GUINEA-PIG LUNG

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SL 85.0324 (2-[[1-(4-fluorophenyl)methyl]-1H-benzimidazol-2-yl-4 piperidinyl]methylamino]-4(3H)- pyrimidinone) is a novel, potent and selective histamine H_1 receptor antagonist (Arbilla et al., 1990) which possesses additional antianaphylactic properties (Lloyd et al., 1990). We decided to examine the effects of SL 85.0324 on thromboxane B_2 (TXB_2) and peptido-leukotriene (LT) release in the isolated perfused guinea pig lungs.

The heart and lungs were excised from anaesthetised male Dunkin Hartley guinea pigs, and the lungs were perfused via the pulmonary artery with oxygenated Kreb's solution containing 0.5% bovine serum albumin (37°C, 5ml/min). After 30 min stabilisation, the perfusates were collected for 5 min (T=0) before the lungs were challenged with 10µg histamine, and the perfusates were collected in 5 min fractions for 15 min. After acidification, eicosanoids were extracted using reverse phase Bond-Elut C_{18} mini-columns and assayed for TXB_2 and peptido-LT using commercially available radioimmunoassay kits (Amersham, France). A separate animal was used for each challenge. T=0 values were subtracted from the post-challenge fractions which were cumulated to give net eicosanoid liberation per challenge.

Control lungs (n=20) challenged with histamine (10µg bolus) released both TXB_2 and peptido-LT (156±28.1 and 1.6±0.4 ng/challenge respectively). SL 85.0324 in the Kreb's (n=5-11 animals per group) dose-dependently inhibited histamine-induced TXB_2 release with significant inhibition in the low nM range, which might be consistent with its anti-H_1 effect. On the other hand, histamine-induced immunoreactive peptido-LT release was only significantly inhibited by SL 85.0324 at concentrations at least an order of magnitude greater than those required to inhibit TXB_2 release. When lungs were challenged with 10µg bradykinin, control TXB_2 release was 738±163 ng per challenge and that of peptido-LT was 3.5±1.1 ng per challenge (n=6). SL 85.0324 even at 100µM had no effect on this release.

We conclude: 1) that SL 85.0324 selectively inhibits the release of bronchoconstrictor eicosanoids induced by histamine which could contribute to its antianaphylactic properties, and 2) that there is a separation between histamine-induced TXB_2 release and LT release which requires further investigation.

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92P STRAIN DIFFERENCES IN THE ANTIBODY RESPONSE: IMPORTANCE OF CORTICOSTEROIDS

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Glucocorticoids are widely used anti-inflammatory and immunosuppressive agents, but the role of endogenous corticosteroids in control of the immune response is less well understood. It has been suggested that the susceptibility of the Lewis rat to experimental diseases such as adjuvant arthritis or autoimmune encephalomyelitis (EAE) may at least be partly due to a dysfunctional hypothalamo-pituitary-adrenal (HPA) axis (Sternberg et al., 1989: Mason et al., 1990). We have studied the humoral response to antigen in four strains of rat with differing susceptibility to such diseases, and investigated the effects of dexamethasone and the steroid antagonist RU486 (mifepristone) in a 'sensitive' (Lewis) and an 'insensitive' (Brown Norway, BN) strain.

Male Lewis, Wistar, Fischer and BN rats (Olac, UK; 250-300g) were injected s.c. with 0.2ml complete Freund's adjuvant containing 0.5mg ovalbumin (OA, Sigma). Dexamethasone sodium phosphate (dex, 0.01mg/kg, s.c.) or RU486 (20mg/kg, p.o.) was administered daily on days 0-4 and 7-11 post-inoculation (p.i.). On day 15 or 21, 50µg OA was injected into the paw and the development of oedema measured plethysmographically. The animals were killed, a blood sample taken and the specific antibody (IgG) titre measured using an ELISA method with OA as the antigen. The paw volume 2h after injection of OA (shown as % increase) and the titre of specific IgG is shown in Table 1.

Table 1: Paw oedema (% increase 2h post injection) and IgG titre: n = 5 rats per group

	15 days, control		15 days, dex		15 days, RU		21 days, control	
	vol	titre	vol	titre	vol	titre	vol	titre
Lewis	27.4 ±3.2	2651 ±725	30.0±3.0	2325 ±543	95.4 ±18.4[b]	7278 ±1332[a]	96.9 ±22.7	8623 ±2381
Fischer	ND	8336 ±2762[c]	—	—	—	—	ND	14439±3041
Wistar	107.5±19.7[d]	14911±4039[c]	—	—	—	—	87.1 ±3.6	21061±4565[c]
BN	115.1±5.6[e]	17815±1609[d]	99.1±7.0*	12068±1553[a]	147.8±9.3[a]	30357±10219	102.9±16.1	18203±2381[c]

a P<0.05, b P<0.01 vs control; * P<0.05 at T = 1h; Student's t. c P<0.05, d P<0.01, e P<0.001 vs Lewis, ANOVA. ND = not determined

The Lewis rat has a significantly lower anti-OA titre than the other strains at 15 and 21 days p.i. and this is reflected in a smaller oedema at day 15, when response is proportional to antibody titre. Dex did not affect the titre in the Lewis; although the titre in the BN was significantly reduced, the paw oedema was significantly smaller only at 30 and 60 min post injection (data not shown). RU486 significantly enhanced both titre and oedema response in the Lewis. In the BN the oedema was increased at 2h and subsequent time points; although the titre was higher, this was not significantly different.

In conclusion, the Lewis mounted a significantly smaller antibody response to OA than did the other strains of rats tested. Endogenous corticosteroids appear to play a role in limiting the antibody response since repeated administration of the antagonist RU486 significantly enhanced the titre in Lewis and tended to elevate it in the BN. In addition, low dose dexamethasone treatment inhibited the titre in the BN but not the Lewis rat. These results are not easily reconciled with the concept of a dysfunctional HPA axis in the Lewis rat although the different effects of RU486 may reflect differing levels of endogenous corticosterone in these two strains.

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93P OESTROGEN RECEPTORS AND ROLE OF AROMATISATION IN TESTOSTERONE-INDUCED INHIBITION OF THYMUS DEVELOPMENT IN FEMALE RATS

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The sex hormone testosterone markedly inhibits thymus development, and this effect might be due, at least in part, to the conversion of testosterone to oestradiol (Greenstein, Mander et al.,1988). Steroid effects are mediated by intracellular receptors, and in this study we attempted to relate the atrophic actions of testosterone to oestrogen receptor activity. Immature female rats weighing 50-65g were implanted s.c. with SILASTIC implants (I.D. 0.45cm; 1.5cm long) containing one of a variety of compounds: testosterone (5 or 20mg); oestradiol (5mg); dihydrotestosterone (DHT) 5mg; the anti-oestrogen tamoxifen (25 or 50mg); the aromatase inhibitor 1,4,6-androstatriene-3,17-dione (ATD 25 or 50mg); the 5-alpha-reductase inhibitor 17-B-N,N-diethyl carbamoyl-4-methyl-4A,5-alpha-androsten-3-one (4-MA) 25 or 40mg) or a combination of testosterone and one of the enzyme inhibitors. Some animals received implants containing cholesterol. On the fourteenth day after implantation the thymuses were removed, weighed and cytosolic estrogen receptors prepared and assayed essentially as described by Ginsburg, Greenstein et al. (1974).

Testosterone and oestradiol at 5mg inhibited thymus growth, although oestradiol was twice as potent as testosterone ($P<0.001$; Duncan's multiple range test). Tamoxifen and 4-MA at 25mg were as potent as 5mg testosterone. ATD was without effect, although it blocked the atrophic action of testosterone.

Tamoxifen and oestradiol caused profound falls (x5) in oestrogen receptor number ($P<0.001$), whereas testosterone produced no change in receptor numbers, which were significantly increased if testosterone was combined with the enzyme inhibitors ($P<0.001$). Aromatisation may be important in androgenic action on the thymus, and may involve effects on oestrogen receptor levels.

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94P MEASUREMENTS OF THYROTROPHIN RELEASING HORMONE AND THE THYROID AXIS IN RATS DURING LEARNING ON A WATER MAZE: EFFECTS OF SCOPOLAMINE

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Cholinergic antagonists produce learning deficits in rats using a water maze (WM; Hagan *et al.*, 1987) and post-training changes in endogenous thyrotrophin-releasing hormone (TRH) levels (Watson *et al.*, 1990). Furthermore, TRH enhances central cholinergic systems which have been implicated in cognition. The aim of this study was to determine the changes in TRH-thyroid axis hormones during WM learning and after treatment with scopolamine (SCP).

Male Hooded Lister rats (230-280 g) were assigned to 4 groups (A to D, n=20 each) as follows; Group A (3 day learning; 60 s free swimming on day 1 and 180 x 2 trials on days 2 and 3); Group B (2 days learning; as Group A minus day 3); Group C ('swim control'; day 1 only) and Group D ('no swim control'; SAL or SCP - 60 min). Thirty min before training on a 2 m diameter WM at 25°C (Watson *et al.*, 1990) rats were treated with either saline (SAL; 1ml/kg i.p., n=10) or SCP (0.5mg/kg i.p., n=10). After testing rats were decapitated and plasma thyroid-stimulating hormone (TSH) and free thyroxine (T_4) levels and TRH levels in the hypothalamus (HYP), septum (SEP), nucleus accumbens (NAc) and medial-parietal cortex (MPC) were determined by radioimmunoassays.

Latency to reach the platform for animals in Group A significantly decreased from trials 1 to 4 (SAL; $P<0.05$, Kruskal-Wallis followed by Multiple Comparisons Test) indicating learning which was significantly inhibited by SCP ($P<0.05$-0.01). In Group B significant learning was not observed from trials 1 to 2 but SCP treated rats took significantly longer to reach the platform.

Although plasma TSH (2.1 ± 0.25 μIU/ml, mean ± s.e. mean, n=10) and T_4 (73.8 ± 7.1 pmol/l, n=10) were not altered by SCP in Group D ('no swim controls') T_4 was significantly elevated ($P<0.05$; ANOVA followed by Dunnett's test) irrespective of drug treatment in both Group A and the 'swim control' (Group C). However, following 2 days learning (Group B), T_4 and TSH were significantly reduced ($P<0.05$) by SCP. TRH levels in saline treated rats (HYP; 504 ± 100, SEP; 270 ± 75, NAc; 101 ± 9 and MPC; 12 ± 5 pg/mg protein, n=6) were not altered by SCP in Group D. HYP TRH levels were reduced in Groups B and C irrespective of drug treatment. NAc TRH levels were significantly reduced in SCP rats from Groups A (40.1 ± 8, $P<0.05$) and B (29.1 ± 4, $P<0.01$).

These results show that training on the WM induces changes in the hypothalamic-thyroid axis which may be associated with the stress of cold exposure. Such changes are not affected by SCP treatment and may, therefore, be unrelated to cognitive performance in the maze.

C.D.W. is a SERC CASE student in conjunction with Reckitt & Colman.

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95P SELECTIVE PHOSPHODIESTERASE INHIBITORS AND THE RELEASE *IN VITRO* OF CORTICOTROPHIN (ACTH) AND LUTEINISING HORMONE

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Previous studies in this laboratory have suggested that two xanthines, caffeine and denbufylline,facilitate the release of ACTH and LH from the pituitary gland by inhibiting a cyclic AMP specific phosphodiesterase (PDE, Hadley et al., 1990).Hence, we have now examined the effects of a number of selective PDE isozyme inhibitors (Beavo et al.,1990) on the resting and hypothalamic extract stimulated release of ACTH and LH from rat anterior pituitary segments in vitro. The tissue was collected and incubated as described previously (Hadley et al., 1990) and exposed to hypothalamic extracts (0.1HE/ml=submaximal concentration) and/or appropriate PDE inhibitors. Hypothalamic extracts readily elicited the release of both hormones.ACTH secretion was unaffected by trifluoperazine (0.1 - 100μM), an inhibitor of type I PDE, but both the basal (0.1 & 1μM, $P<0.05$) and HE-stimulated (0.1μM, $P<0.01$;1-100μM, $P<0.05$) release of LH were reduced. Milrinone (0.1-100μM), which inhibits type III PDE, had no effect on basal hormone release but attenuated both the ACTH ($P<0.05$) and LH (0.1-10μM,$P<0.01$) responses to HE stimulation. Zaprinast (0.1-10μM), a cyclic GMP specific (type V) PDE inhibitor, had little effect on the secretion of ACTH although at the highest concentration it produced a small increase in basal release. In contrast, it effectively blocked the HE-elicited release of LH ($P<0.01$) and tended to reduce the basal release of the hormone. Denbufylline (0.01-1mM), which blocks the cyclic AMP specific cyclic GMP independent (type IV) PDE, precipitated significant ($P<0.05$) increases in ACTH release while at lower concentrations (0.1-1μM) it potentiated markedly ($P<0.01$) the responses to HE. Similar but less pronounced effects on LH secretion were apparent. Another type IV PDE inhibitor, rolipram (0.1-100μM), also increased resting ACTH release (100μM, $P<0.05$) and potentiated the corticotrophic response to HE (100μM, $P<0.01$) but, unlike denbufylline, had no effect on LH release. Similarly, a non-selective PDE inhibitor, papaverine (0.1-100μM), produced small but significant ($P<0.01$), concentration dependent increases in resting and HE induced ACTH release ($P<0.05$) but had no effect on LH secretion. Forskolin (0.1μM-1mM), which activates adenylate cyclase, increased ACTH and, to a lesser extent, LH release; its effects were potentiated by denbufylline (1 & 100μM, $P<0.05$).The results provide further evidence that xanthines such as denbufylline facilitate ACTH release by inhibiting the cyclic AMP specific cyclic GMP independent (type IV) PDE. They also support suggestions that cyclic AMP may contribute to certain aspects of LH release and provide novel evidence for an inhibitory role for cyclic GMP in control of this pituitary hormone.

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96P THE EFFECT OF ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE AND GUANINE NUCLEOTIDES UPON CALCIUM-EVOKED ACTH SECRETION FROM ELECTRICALLY PERMEABILISED ATT-20 CELLS

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The stimulation of ACTH secretion, by a variety of agents, from AtT-20 anterior pituitary cells is mediated by adenosine 3',5'-cyclic monophosphate (cyclic AMP) which promotes secretion by both enhancing voltage-dependent calcium entry and potentiating the effect of calcium upon the secretory apparatus (Guild et al., 1986). The present study investigated whether cyclic AMP may exert an action at the level of a GTP-binding protein implicated in mediating the effects of calcium upon ACTH secretion in AtT-20 cells (Luini & De Matteis, 1988).

The effects of cyclic AMP, guanosine 5'-O-(3'-thiotriphosphate) (GTP-γ-S, 10^{-4}M) and guanosine 5'-O-(2-thiodiphosphate) (GDP-β-S, 10^{-4}M) upon calcium (10^{-9}-10^{-3}M)-evoked ACTH secretion from electrically permeabilised cells was investigated using methods previously described (Guild et al., 1988). The effect of cyclic AMP (10^{-4}M) upon GTP-γ-S (10^{-8}-$3x10^{-4}$M)-stimulated ACTH secretion, in the absence of calcium, from electrically permeabilised cells was also studied. ACTH levels were measured by radioimmunoassay (Reisine, 1984).

Calcium-stimulated ACTH secretion (251±10pg/10^6 cells/20 min at maximum) was concentration-dependent (EC_{50} = $7x10^{-7}$±$1x10^{-7}$M, n=3). Cyclic AMP (10^{-4}M) potentiated calcium-evoked secretion (2-fold at maximum) without significantly altering the EC_{50} for calcium (EC_{50} = $5x10^{-7}$M ± $1x10^{-7}$M, n=3). GTP-γ-S (10^{-4}M) stimulated an ACTH secretion of 180±10pg/10^6 cells/20 min at a calcium concentration of 10^{-9}M and was additive with calcium-evoked secretion up to a maximal stimulation of 255±10pg/10^6 cells/20 min) which could be achieved by calcium (10^{-5}M or above) alone. Calcium (10^{-5}M)-evoked ACTH secretion was inhibited 80% by GDP-β-S (10^{-4}M). GTP-γ-S (10^{-6}-$3x10^{-4}$M) stimulated ACTH secretion was potentiated (2-fold at maximum) by cyclic AMP (10^{-4}M).

The results of the present study support the hypothesis that cyclic AMP is acting to modulate the ability of changes in cytosolic free calcium to stimulate secretion. A target for regulation by cyclic AMP may be a GTP-binding protein which may mediate the effects of calcium upon the secretory apparatus.

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97P THE EFFECTS OF DRUGS AND DE-MEDULLATION ON PRESSOR RESPONSES TO PREGANGLIONIC NERVE STIMULATION IN THE PITHED RAT

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There is evidence that the vasopressor response to stimulation of the sympathetic outflow in the pithed rat is partly adrenergic and partly purinergic (Bulloch & McGrath, 1988). This study re-examined this hypothesis and considered the role of the adrenal medulla and the contribution of angiotensin II to this response. Male Wistar rats (300-350g) were anaesthetised with trichlorethylene, pithed (Gillespie et al, 1970), respired artificially with air and given gallamine (10mg kg^{-1}, i.v.). Blood pressure was recorded from the right carotid artery. Drugs were administered via the right femoral vein. The pithing rod electrode was used to stimulate the sympathetic outflow at T_8 (1cm electrode, supramaximal voltage, 0.4ms pulses 2-20Hz). Stimulation at 2 and 20Hz (100 pulses) produced characteristic pressor responses during stimulation and prolonged responses after stimulation. De-medullation of the adrenals substantially reduced the pressor response that occurred during stimulation by 50-70% and abolished the pressor response that persisted after stimulation. A large component of the pressor response was therefore adrenal in origin. In separate experiments propranolol (2mg Kg^{-1}, i.v.) was administered to block the vasodilation caused by catecholamines released from the medulla. The effect of propranolol was variable and in some rats potentiated pressor responses to nerve stimulation or noradrenaline (NA) but in others the pressor responses were inhibited by propranolol. This was surprising since propranolol had been expected to block vascular β_2-adrenoceptors and only to potentiate the pressor responses. Instead, in some rats, propranolol may have blocked the effect of catecholamines in the heart. Since endogenous angiotensin II potentiates adrenergic responses in the pithed rat (Vollmer et al, 1984), the effects of captopril (1mg kg^{-1}) were examined. Captopril had little effect on the resting blood pressure but inhibited responses to NA by 46± 4% (mean ± s.e. mean, n=12, P<0.001) and to nerve stimulation at 2Hz by 52±5% (n=12, P<0.001) and at 20 Hz by 41±6% (n=12, 0.01>P>0.001). Prazosin (2mg kg^{-1}) administered after captopril usually abolished the residual responses to NA and nerve stimulation. Occasionally, when the residual response to nerve stimulation was not abolished by prazosin, desensitisation with, α,β-methylene ATP (Bulloch & McGrath, 1988) had little additional effect. These results underline the complexity of this preparation and the difficulties likely to be encountered in studying co-transmission in it.

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98P SYMPATHETIC NEUROEFFECTOR TRANSMISSION IN THE RABBIT ISOLATED SAPHENOUS VEIN

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The rabbit isolated lateral saphenous vein has a heterogeneous population of post-junctional α-adrenoceptors ($\alpha_2>\alpha_1$) (Daly *et al.*, 1988). In the present study we have examined the effects of antagonists versus sympathetic nerve stimulation to determine the contribution of these receptor subtypes during neuromuscular transmission. The presence of pre-junctional α_2-adrenoceptors on this preparation has already been demonstrated (Levitt & Hieble, 1986).

Male New Zealand White rabbits (2-3 Kg) were killed by stunning followed by exsanguination. Saphenous veins were prepared as previously described (Daly *et al.*, 1988) and rings (3-4mm in length) were then suspended between parallel platinum electrodes for stimulation. After a 1 hour equilibration period frequency response curves (FRCs) were constructed (Square 1 instruments stimulator, 4-64 Hz, 0.1ms pulse width, 35 volts, 1 sec. train duration). After an initial FRC, ancillary drugs or antagonists were added to the bath and allowed at least 20 minutes to equilibrate before the next FRC.

Responses were unaffected by propranolol (1μM) or corticosterone (30μM). Cocaine (10μM) increased response height and duration. In particular, responses at 32Hz & 64Hz developed a visible secondary response. The size of this secondary component varied; it could be greater than the initial component or could appear as a 'shoulder' on the response. The initial, fast, component (*I*) peaked at 4-6 seconds and the secondary, slow, component (*II*) was maximal at 18 seconds. Selective concentrations, judged by their effects on agonists in this preparation, of rauwolscine (1μM), CH 38083 (1μM), corynanthine (1μM) and prazosin (0.1μM) were tested against the biphasic responses obtained in the presence of cocaine (10μM) & propranolol (1μM). Rauwolscine was found to inhibit *II* (10-50%) while increasing the size of *I* (0-40%). CH 38083 was found to inhibit *II* (74-86%) while increasing the size of *I* (0-28%). Prazosin reduced the height of both *I* (85-90%) & *II* (85-90%) leaving a slow response. Corynanthine also reduced both *I* (25-55%) & *II* (45-60%) leaving a smaller fast response. The combined effect of prazosin and rauwolscine was not sufficient to abolish the response.

The results suggest that both phases of the response to sympathetic nerve stimulation involve α_1-adrenoceptors. It seems clear that the initial fast component (*I*), which is not blocked by rauwolscine, is mediated through α_1-adrenoceptors. Rauwolscine's potentiation of *I* is probably due to increased transmitter release via blockade of pre-junctional α_2-adrenoceptors. In the presence of uptake$_1$ blockade, causing a persistence of NA, the slow component (*II*) which arises is sensitive to rauwolscine or CH 38083 and is therefore mediated in part through α_2-adrenoceptors. However very effective blockade of *II* by prazosin and corynanthine suggests that the α_2 response requires facilitation from α_1-adrenoceptors, just as prazosin inhibited responses to "selective" α_2-adrenoceptor agonists in this tissue. We conclude that both α_2 and α_1 receptor subtypes can contribute to neuromuscular transmission although $\alpha_1>\alpha_2$; for exogenous NA however it is the reverse ($\alpha_2>\alpha_1$) (Daly *et al.* 1988). Furthermore the preparation is suitable for the demonstration of the simultaneous participation of pre- and post-junctional α_2-adrenoceptors during sympathetic neurotransmission.

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99P ENDOGENOUS NITRIC OXIDE INHIBITS ADRENERGIC NEUROGENIC VASOCONSTRICTION IN GUINEA-PIG PULMONARY ARTERY

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Although it is well documented that the vascular endothelium exerts an inhibitory influence on the contractile response to a variety of stimuli, the exact mechanism underlying this inhibition is still unclear. We have, therefore, explored the possible inhibitory roles of endogenous prostacyclin (PGI_2) and nitric oxide (NO) on adrenergic neurogenic vasoconstriction.

The two branches of the pulmonary artery were dissected from Dunkin-Hartley guinea-pigs, and cut into rings, 2 mm in length, then suspended in 2-ml organ baths. Changes in isometric tension were recorded. Field stimulation was applied using a Grass S88 stimulator via a pair of platinum electrodes positioned on either end of the vessel rings.

Field stimulation (EFS, 50v, 0.2 ms duration, 1-16 Hz, 15s) induced a frequency-dependent contraction, which was abolished by tetrodotoxin (1μM) and by phentolamine or prazosin (each 1μM), indicating that this contraction was due to the activation of α_1-adrenoceptors by noradrenaline (NA) released from sympathetic nerves. Although indomethacin (1-10μM) had no significant effect on either EFS- or NA-induced contraction, N^G-monomethyl L-arginine (L-NMMA) caused a 1+0.2, 1.7+0.3, 2.7+0.4 and 3.4+0.5-fold increase in the EFS-induced contraction at concentrations of 3, 10, 30 and 100 μM respectively (n=5, $p < 0.01$ compared with control group). The augmenting effect of 30μM L-NMMA on EFS contractions was completely reversed by 100-300μM L-arginine, but not by an identical concentration of D-arginine. The contractile response to exogenous NA was also enhanced by 30μM L-NMMA (pD_2 values, 6.4+0.2 vs 7.1+0.4, $p < 0.01$, n=5). Contractions elicited by exogenous administration of NA (0.1μM), phenylephrine (PE, 0.1μM) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 0.6μM) which matched the EFS-induced (4 Hz) contraction were equally augmented (2.9+0.6, 2.8+0.6, 3.1+1.0 and 2.9+0.6-fold increase for NA, PE, $PGF_{2\alpha}$ and EFS contractions respectively) by 30μM L-NMMA, whereas $PGF_{2\alpha}$ and PE had no relaxant effects and NA had little relaxant effect even on the precontracted vessel rings. In U44069 (a thromboxane A_2 analogue)-precontracted vessel rings UK14304, a selective α_2-adrenoceptor agonist, induced a concentration-dependent relaxation, which was abolished by endothelial removal and significantly inhibited by 30μM L-NMMA.

Our results indicate that α_2-adrenoceptors are present in guinea pig pulmonary artery but they seem unlikely to contribute to the inhibitory role of endothelium on NA contraction. Basal and stimulated release of endogenous nitric oxide from vascular endothelium, probably by shear stress imposed on endothelial cells by smooth muscle contraction, inhibits adrenergic neural vasoconstriction, whereas endogenous PGI_2 is not involved in modulating this contractile response.

100P DIFFERENCE IN THE RESPONSE OF TWO AGONISTS AT INHIBITORY PREJUNCTIONAL MUSCARINIC RECEPTORS IN THE RABBIT EAR ARTERY

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Vasoconstrictor responses to nerve stimulation at 3 Hz in the rabbit isolated ear artery, perfused with McEwen's solution containing cocaine (10 μM) and yohimbine (1 μM), are inhibited by carbachol or McN-A-343 acting on prejunctional inhibitory muscarinic receptors.

Previous work has established that the responses to the agonists are inhibited to a similar extent by atropine but McN-A-343 is inhibited to a greater extent by pirenzepine (Choo *et al.*, 1985). Current studies with AF-DX 116, a compound with high affinity for muscarine M_2 receptors, showed that it was a more potent inhibitor ($P < 0.01$) of responses to carbachol than of those to McN-A-343; at a concentration of 5 μM of AF-DX 116 the dose ratios obtained were 70.8 (35.5 - 138) (geometric mean, 95% confidence limits) (n = 4) and 15.9 (8.0 - 30.4) (n = 9) respectively. These findings suggest that the two agonists act at different sites. To explore this possibility further the effects of a number of compounds on the responses to the two agonists were compared. The responses to either agonist were not significantly ($P > 0.05$) affected by indomethacin (100 μM) or by pertussis toxin pretreatment (300 ng/ml for 90 min). While (-) cocaine has some muscarinic receptor blocking activity (Sharkey *et al.*, 1988) (±) cocaine (10 μM) did not affect responses to either agonist in preparations perfused with McEwen's solution containing only yohimbine (1 μM).

ω-Conotoxin GVIA (0.01μM) decreased responses to nerve stimulation to 51.8 ± 3.88% (mean ± s.e.mean) (n = 6) of control responses and caused a small rightward shift in the concentration-response curves of both carbachol and McN-A-343. The dose ratios were 1.95 ± 0.62 (mean ± s.e.mean) (n = 3) and 1.21 ± 0.43 (n = 3) respectively. The difference was not significant ($P > 0.05$).

In conclusion, while the muscarinic receptor antagonist AF-DX 116 differentiated between the two agonists no evidence was found for a different mechanism of action by the use of a cyclo-oxygenase inhibitor, indomethacin, a neuronal N-type calcium channel blocker, ω-conotoxin GVIA or after pretreatment with pertussis toxin.

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101P PHARMACOLOGICAL AND ELECTRON MICROSCOPICAL EXAMINATION OF A BLOOD VESSEL EXHIBITING CO-TRANSMISSION

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Stimulation of the sympathetic nerves to some blood vessels releases more than one transmitter, for example NA and ATP as found in the rabbit ileocolic artery (Bulloch & Starke, 1990).The degree of purinergic involvement in nerve mediated contractile responses in isolated blood vessels varies according to blood vessel size (in general, the smaller the vessel, the larger the purinergic component (McGrath et al, 1990)) and method of stimulation.

For pharmacological studies, ring segments of rabbit ileocolic artery were placed in physiological salt solution maintained at 37°C and gassed with 95%O_2 :5%CO_2. Isometric contractile responses to electrical field stimulation (4-32 Hz, 25-40 V, 0.1 ms pulse width, for 1 s and 20 s) were recorded under a resting tension of 0.5 g wt. Ring segments of the same ileocolic artery were also processed for electron microscopy using a glutaraldehyde osmium tetroxide fixative (Sabatini et al, 1963) and Tranzer's dichromate fixative (Tranzer & Richards, 1976).

Electrical field stimulation produced frequency and pulse number dependent vasoconstrictor responses. At pulse trains over 20s duration two phases can be differentiated. The first one (and the only one detected at stimulations of 1 second duration) was significantly reduced by α,β -methylene ATP (3μM). The second component was only sensitive to prazosin (0.3μM) at high frequencies. The proximal section of the artery has a wall diameter of approximately 80μM (n=5). The tunica intima is composed of a single layer of endothelium overlying the fenestrated elastic lamina. The tunica media has approximately 18 layers of circularly arranged smooth muscle which are connected with nexuses. The adventitia comprises longitudinal elastic and collagen bundles. Unmyelinated nerve bundles (sympathetic) are found principally in the adventitial layer with a few located between the smooth muscle cells in the outer 2 -3 layers of the tunica media.

Since the dominant component of the nerve-mediated response is purinergic, this study supports the idea that at least in the rabbit ileocolic artery purinergic transmission may propagate more readily than noradrenergic transmission through vascular smooth muscle.

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102P EFFECT OF ENDOTHELIN-1 ON HUMAN UMBILICAL AND PLACENTAL BLOOD VESSELS: A COMPARISON WITH 5-HT

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Maternal plasma endothelin-1 (Et-1) levels are increased during labour and delivery and are high in amniotic fluid (Usuki *et al.*, 1990). Here, the potential role of Et-1 as a modulator of fetoplacental vascular resistance has been evaluated.
Umbilical and placental vessels from fresh, full term placentas from uncomplicated pregnancies were studied. Successive branches of decreasing diameter were dissected free and cleaned, then cut into rings (2-3mm width) and suspended in organ baths containing Krebs saline at 37°C under a force of 1.5 g. Arteries were gassed with 2.5% O_2, 8% CO_2 balance N_2 (O_2 tension=16 mmHg) and veins were gassed with 8% O_2, 5% CO_2 balance N_2 (oxygen tension = 55 mmHg). Smaller sub-chorionic100-180 μm stem villi arterioles were mounted, under tension, on a Mulvany-Halpern myograph under the same conditions as described for the larger arteries. Dose-response curves were constructed to Et-1(Bio Mac) and compared with those elicited by 5HT (Sigma). The results are summarised in Table 1.

Table 1 -Log concentration of the EC_{50} and maximum responses to Et-1 and 5HT in placental and umbilical blood vessels

	-Log concentration of the EC_{50}		Maximum response (tension [mg wt])		n
	Et-1	5HT	Et-1	5HT	
Umbilical artery	8.1 ± 0.1	7.2 ± 0.1**	484 ± 146	1081 ± 141*	6
Umbilical vein	9.2 ± 0.5†	7.3 ± 0.5*	865 ± 366	1188 ± 450	4
1° Surface chorionic plate artery	8.0 ± 0.1	6.8 ± 0.1***	492 ± 118	882 ± 250	6
2° Surface chorionic plate artery	7.9 ± 0.1	6.9 ± 0.1***	461 ± 51	450 ± 146	6
3° Surface chorionic plate artery	7.7 ± 0.1	7.1 ± 0.2 **	275 ± 88	108 ± 55	4
3° Surface chorionic plate vein	8.4 ± 0.1††	7.5 ± 0.1***	580 ± 194	339 ± 185	4
2° Stem villus arteriole	8.2 ± 0.2	1.9 ± 0.4***	460 ± 65	64 ± 46***	7

Unpaired Student's *t* test: Vein vs. relevant artery † $P<0.05$, †† $P< 0.01$; 5HT vs. Et-1 *$P<0.05$, **$P<0.01$, ***$P<0.001$

Table 1 shows that for each artery tested, Et-1 was a potent agonist, some 10 times more potent than 5HT and that it was some 10 times more potent again on the veins tested. The potency of Et-1 did not significantly alter with vessel size. While the maximum response to 5HT was significantly greater than that to Et-1 in the umbilical artery, it declined with the vessel size, being significantly less than that to Et-1 in the stem villi arterioles. The maximum response to Et-1 did not significantly alter with vessel size.

These results show that Et-1 is a potent agonist at all levels of the fetoplacental circulation and suggest that it may modulate fetoplacental circulation and assist in its vasoconstriction at birth.

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103P EFFECTS OF FLECAINIDE IN VASCULAR SMOOTH MUSCLE

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Flecainide acetate is a class Ic antiarrhythmic drug which inhibited the slow action potentials elicited by isoprenaline in K-depolarized ventricular muscle fibres (Schulze & Knops, 1982) and prolonged the relative and functional refractory periods of the atrioventricular node (Somberg & Tepper, 1986). These results suggest that flecainide may exhibit calcium antagonistic properties in cardiac muscle. In the present study the effects of flecainide on contractile responses and ^{45}Ca uptake were studied in isolated rat aortic rings and portal vein segments.

Aortic rings (2-3 mm in length) and portal vein segments from rats were incubated in Krebs-Henseleit solution (KS). Techniques employed to estimate ^{45}Ca uptake in aortic rings were as previously described by Barrigón & Tamargo, 1986.

In aortic rings flecainide, 10^{-6}M-10^{-4}M, inhibited the contractions induced by 80 mM K and 10^{-5}M noradrenaline (NA), the IC_{50} values for flecainide depression being $3.5 \pm 0.8 \times 10^{-5}$M and $8.3 \pm 0.5 \times 10^{-5}$M, respectively. Contractions induced by addition of Ca (1-5 mM) to 0Ca high K KS were also inhibited by flecainide ($IC_{50} = 2.5 \pm 0.3 \times 10^{-5}$M). The effects of flecainide were also studied on the phasic contractile responses induced by NA in rings incubated in 0Ca KS, then in 2.7 mM Ca (Ca-loading) and finally in 0Ca KS. Flecainide, 10^{-5}M and 10^{-4}M, when applied after Ca-loading inhibited by 26.4 ± 7.3% ($P < 0.05$) and 56.1 ± 4.9% ($P < 0.01$), respectively, the magnitude of NA-induced phasic contraction. Flecainide 10^{-4}M, had no effect on ^{45}Ca uptake in resting aortic rings but significantly decreased ^{45}Ca uptake stimulated either by NA (from 0.578 ± 0.037 to 0.427 ± 0.027 mmol/kg aorta, $n = 7$, $P < 0.05$) and KCl (from 0.657 ± 0.067 to 0.390 ± 0.027 mmol/kg aorta, $n = 7$, $P < 0.05$). Flecainide also inhibited the amplitude of the spontaneous myogenic activity in portal vein segments ($IC_{50} = 6.5 \pm 0.9 \times 10^{-5}$M).

These results suggested that in rat isolated aortae and portal veins flecainide inhibited Ca entry through voltage-operated channels and NA-induced Ca uptake as well as Ca release from intracellular stores.

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104P INTERACTIONS OF PHENELZINE WITH VASOCONSTRICTOR AND VASODILATOR MECHANISMS IN RAT AORTA

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The relaxation of arterial smooth muscle by acetylcholine (ACh) involves an endothelium-dependent relaxant factor (EDRF) (Furchgott and Zawadzki, 1980). Griffith *et al* (1984) showed that phenylhydrazine reversed the relaxant effects of ACh on endothelium-intact rabbit aorta without directly affecting tone in endothelium-denuded vessels. They proposed that EDRF contained a carbonyl group which reacted with phenylhydrazine to inactivate EDRF, although recent studies now suggest that EDRF may be the non-carbonyl compound nitric oxide (*e.g.* Palmer *et al.*, 1987). The structural similarity between phenylhydrazine and phenelzine (2-phenylethylhydrazine), a monoamine oxidase (MAO) inhibitor in clinical use as an antidepressant agent, prompted us to carry out related studies with phenelzine (PHEN) upon rat aorta.

Thoracic aortic rings from male Sprague-Dawley rats (350-500g) were suspended for isometric recording between parallel stainless steel wires under 1g tension in organ baths containing modified Krebs' bicarbonate buffer gassed with 5% CO_2 95% O_2 at 37°C, pH 7.4. Preparations were contracted with 0.1 μM noradrenaline (NA), and then relaxed by addition of 1.0 μM ACh to establish the presence of a functional endothelium. Addition of PHEN (1, 3, 10, 30, or 100 μM) at the point of maximal relaxation produced a progressive reversal of relaxation in a concentration-dependent manner, while relaxations were maintained in preparations not treated with PHEN. Reversal of ACh relaxations were also obtained when KCl (35 mM) rather than NA was used as the initial contractile agent, suggesting that these effects of PHEN were not simply due to potentiation of NA by MAO inhibition. Ibuprofen (10 μM) did not modify the actions of PHEN suggesting that endogenous prostanoids formation was not involved. In endothelium-denuded (rubbed) rings, 30 μM PHEN reversed sodium nitroprusside (10 nM) relaxations in KCl (35 mM) contracted tissues. All these actions of PHEN were removed after its washout from the tissues. PHEN alone (30-100 μM) had no direct contractile effects on preparations unexposed to other agents. During cumulative addition of KCl (15 - 95 mM), pretreatment with 10, 30 or 100 μM PHEN potentiated the contractile effects of low (15 - 35 mM) KCl concentrations in both rubbed and unrubbed preparations, a similar result to that obtained using the Ca agonist BAY K-8644 (30 - 100 nM) as pretreatment. Also, 30 μM PHEN enhanced the contractile effects of adding $CaCl_2$ (0.1 - 2.5 mM) to tissues depolarized with 35 mM KCl in Ca-free buffer.

In summary, PHEN has vasoconstrictor actions unrelated to MAO inhibition, which are not necessarily dependent upon interactions with EDRF, and which may involve blockade of hyperpolarizing potassium efflux and/or enhancement of Ca availability in smooth muscle by mechanisms which require further investigation.

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105P INTERACTION BETWEEN EXTRACELLULAR CALCIUM, 5-HT, THROMBOXANE A_2 AND OXYGEN IN THE HUMAN UMBILICAL ARTERY

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We have previously demonstrated the requirement of endogenous thromboxane (TxA_2) to 'uncover' a 5-HT_1-like receptor in isolated strips of human umbilical artery (Templeton et al, 1991). The involvement of extracellular calcium in this effect has been examined in this study by the use of the calcium channel facilitator Bay-K 8644 and the channel blocker nifedipine. We have also examined the effects of both of these drugs on contractions to oxygen *per se* and U46619 (TxA_2 stable analogue).

Strips of human umbilical artery (1-1.5cm) were set up in Krebs at 37°C bubbled with 2.5% O_2/ 8% CO_2 balance N_2 (O_2= 16mmHg) (for methods see MacLennan et al 1988). Cumulative concentration response curves were constructed to either 5-HT or U46619 in the presence of nifedipine(0.1-1μM) or Bay-K(0.01-0.1μM). In the case of oxygen non-cumulative curves were constructed. When 5-HT was used as the agonist, the curves were repeated in 16% O_2/ 8% CO_2 balance N_2 (O_2=120mmHg). To demonstrate 5-HT_1-like receptors seen only in 16% oxygen methiothepin was used against 5-CT(5-carboxyamidotryptamine) and 5-HT in some experiments.To separate the effect of Bay-K on responses mediated by different 5-HT receptors, responses to 5-HT in 2.5% oxygen were repeated in the presence of ketanserin (30nM) or methiothepin (1nM).

Nifedipine(1μM) significantly inhibited maximum responses to 5-HT at both oxygen tensions (2.5%,16%) and to oxygen *per se* by 43%, 48% & 45% respectively. Responses to U46619 were significantly inhibited at low concentrations, e.g control 3nM; 890±190 1μM nifedipine 440±80(mg.wt), but not at the maximum. Bay-K (0.01-0.1μM) invariably induced a maintained contraction; superimposed responses to U46619 were potentiated at the lower concentrations, with a reduced maximum, but this could be attributed to the raised baseline rather than a depressant effect of Bay-K. Bay-K potentiated responses to oxygen and decreased the threshold concentration for contraction to 5-HT in 2.5% O_2 resulting in a 5-HT concentration response curve similar to that in 16% O_2. This 'extra' response was similar to the effect of a low concentration of U46619 (0.3nM) in the presence of flurbiprofen (0.1μM) to inhibit basal TxA_2 production. Thus, Bay-K 8644, like the stable TxA_2 analogue, can mimic the synergistic action of oxygen, uncovering a response via 5-HT_1-like receptors.

Thus it seems that facilitating the opening of calcium channels with a dihydropyridine agonist produces the same effect as exogenous or endogenous TxA_2, resulting in both contraction *per se* and facilitation of a 5-HT_1-like receptor response. Overall it seems that dihydropyridine-sensitive calcium channels in this preparation can be activated via various receptors and can play a facilitating role in receptor-mediated contraction of smooth muscle, which merely enhances responses via TxA_2 receptors but is essential to underpin responses via 5-HT_1-like receptors.

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106P FURTHER INVESTIGATIONS OF CONTRACTILE RESPONSES TO 5-HT IN THE HUMAN SAPHENOUS VEIN

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5-Hydroxytryptamine (5-HT) produces contractions of the human saphenous vein by action at both 5-HT_2 and 5-HT_1-like receptors (Docherty & Hyland, 1986; Borton & Docherty, 1988). We now report our further studies of the 5-HT_1-like receptors of human saphenous vein.

Human saphenous veins were obtained following varicose vein surgery and ring preparations of side branches of the main saphenous veins were employed in investigations of isometric contractions. The interactions between agonists and antagonists were investigated, and all effects of antagonists were corrected for changes occurring in vehicle experiments.

The 5-HT_2 receptor antagonist ketanserin (1μM) produced non-parallel shifts in the concentration-response curve (CRC) to 5-HT, shifting mainly the upper part of the CRC, so that the lower part of the CRC to 5-HT is mediated by 5-HT_1-like receptors. Metitepine (0.1μM) produced an approximately parallel shift in the CRC to 5-HT, whereas cyanopindolol (10μM) failed to shift the potency of 5-HT. Metergoline (0.1μM) and cyproheptadine (0.1μM) shifted the lower part of the CRC to 5-HT, but produced larger shifts in the upper part of the CRC. The pK_B values (and 95% confidence limits) obtained against the lower part of the CRC to 5-HT were 7.91± 0.69 (n=5) and 7.86 ± 0.21 (n=3) for metergoline and metitepine, respectively. The agonists 5-HT, 5-carboxamidotryptamine and sumatriptan contracted the human saphenous vein with pD_2 values of 6.33 ± 0.18, 5.99 ± 0.38 and 5.16 ± 0.70.

In conclusion, based on the potency of metergoline, the contractile 5-HT_1-like receptor of the human saphenous vein may resemble the 5-HT_{1D} site as has been suggested for the contractile 5-HT_1 receptor of rabbit saphenous vein (Martin et al., 1991), although further investigations are required to establish this.

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107P THE APPARENT HETEROGENEITY IN FUNCTIONAL 5-HT_2 RECEPTORS REVEALED BY ANTAGONISTS MAY BE SPECIES-RELATED

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A previous analysis of the 5-HT_2 receptors which mediate contraction of rabbit aorta, rat jugular vein and rat caudal artery preparations showed that the antagonists conventionally used to define this receptor type demonstrate variable affinities in the different tissues studied (Leff & Martin, 1986). These results, together with discrepancies between affinity estimates reported by other laboratories, led to the conclusion that either genuine 5-HT_2 receptor heterogeneity might exist or that the antagonists used were unreliable. A more recent review of published data from various laboratories (Mylecharane, 1990) indicated that affinity (pK_B) values for ketanserin comprised two sub-groups; in rat jugular vein, caudal artery, femoral artery and uterus preparations affinities tended to be higher than those in other rat tissues (aorta and portal vein) and in rabbit, guinea-pig and dog smooth muscle preparations The limited available data suggested a similar pattern for methysergide, trazodone and LY53857, but not for spiperone which was consistently higher in rat preparations. Subtle variations in experimental conditions and analysis in different laboratories can either mask genuine differences in pK_B values, or reveal apparent differences which are spurious. To minimize this potential complication, investigations of the antagonist actions of spiperone, trazodone and LY53857 have now been completed in rat aorta (resting tension 1.0 g, in the presence and absence of 30 μM (±)cocaine) as well as in rabbit aorta, rat caudal artery and rat jugular vein, using the same experimental conditions and analytical methods described Leff & Martin (1986). Tissues were pretreated with pargyline (500 μM) and benextramine (10 μM) for 30 min to induce irreversible inhibition of monoamine oxidase and α_1-adrenoceptors. One cumulative concentration-effect curve for 5-HT was obtained from each preparation, in the absence or presence of antagonist added 60 min prior to commencement of the 5-HT curve. The results are summarized in Table 1.

Table 1. pK_B values and Schild plot slopes in parentheses (means ± s.e.mean; n = 3 - 6 at each antagonist concentration tested; *Leff and Martin, 1986)

Antagonist	Rat aorta	Rat aorta (cocaine)	Rat caudal artery	Rat jugular vein	Rabbit aorta
Spiperone	9.27±0.10 (0.98±0.07)	9.64±0.21 (1.23±0.13)	9.63±0.09 (0.91±0.07)*	9.66±0.11 (0.98±0.14)*	9.28±0.10 (1.10±0.06)*
Trazodone	7.58±0.10 (0.90±0.07)	7.86±0.10 (1.12±0.07)	8.32±0.12 (0.91±0.08)*	8.02±0.07 (0.90±0.08)*	7.23±0.19 (1.00±0.13)*
LY53857	7.75±0.17 (0.88±0.22)	7.78±0.16 (0.96±0.21)	non-surmountable	non-surmountable	7.43±0.14 (0.81±0.11)

These results suggest that in rat aorta adrenergic neuronal uptake of 5-HT leads to underestimation of pK_B values for spiperone and trazodone, but not LY53857. This unexpected complexity with LY53857, and its tendency to demonstrate non-surmountable antagonism in some tissues, clearly limits its utility in the present analysis. However, the apparent distinction between the receptors in rat aorta and the other rat tissues suggested by trazodone and spiperone in the absence of (±)cocaine can be explained by the practical conditions used; when account is taken of agonist removal mechanisms pK_B values for spiperone and trazodone are similar in each of the rat tissues studied, but significantly higher than those obtained in rabbit aorta. This might suggest that apparent heterogeneity in functional 5-HT_2 receptors observed with some antagonists is species-dependent.

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108P FENOLDOPAM (SKF 82526) IS A LOW EFFICACY AGONIST AT THE 5-HT_2 RECEPTOR

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The selective DA_1-receptor agonist fenoldopam (FNP) is also a 5-HT_2 agonist in canine tracheal smooth muscle (Gretler et al, 1990). To fully characterise the activity of FNP at the vascular 5-HT_2 receptor, we examined its properties using endothelium-denuded rabbit aortic rings (Leff & Martin, 1986).

Rabbit aortic rings (2-3 mm wide) were suspended in Krebs solution (95% O_2 : 5% CO_2, 37 °C) and equilibrated at a resting tension of 2g to measure isometric tension changes. The rings were contracted with an effective dose of 5-hydroxytryptamine (5-HT, 10 μM) and discarded if relaxation to acetylcholine (ACh, 1 μM) occurred. After pretreatment with benextramine (10 μM) and indomethacin (3 μM) to block α_1-adrenoreceptors and cyclo-oxygenase respectively, cumulative dose-response curves to 5-HT or FNP were constructed 45 minutes after the addition of either saline, ketanserin or (in the full/partial interaction study) FNP. Tissue responses were expressed as a percentage of the initial siter response to 5-HT and the EC_{50} and intrinsic activity (I.A., relative to 5-HT) calculated. pA_2 values for ketanserin and FNP were determined by Schild analysis (Schild, 1947). Results are expressed as mean ± s.e.mean.

Comparison of 5-HT and FNP as 5-HT_2 receptor agonists

	pEC_{50}	I.A.	n
5-HT	7.04 ± 0.06	1	8
FNP	5.53 ± 0.13	0.44 ± 0.05	8

The constrictor effects of 5-HT and FNP were competitively antagonised by ketanserin with pA_2 values against 5-HT and FNP of 8.74 and 8.87 respectively (pooled data from 3-4 experiments). In the full-partial interaction study, FNP produced a dose-related rightward shift of 5-HT dose-response curves with a pA_2 value of 5.35 ± 0.11 (slope 1.07 ± 0.08) together with some non-competitive blockade, as revealed by a reduction in the maximum response to 5-HT at FNP concentrations > 100μM (n = 6).

The results of this study indicate that, in addition to being an agonist at peripheral DA_1-receptors (Hahn et al, 1982) and an antagonist at α_2-adrenoreceptors (Ohlstein et al, 1985), FNP is a low efficacy agonist at the vascular 5-HT_2-receptor.

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109P DEMONSTRATION OF POST-JUNCTIONAL α_2-ADRENOCEPTORS IN PERFUSED RABBIT EAR ARTERY

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Demonstration of post-junctional α_2-adrenoceptors in vivo (Docherty,J.R. & McGrath,J.C., 1980; McGrath,J.C. 1982) precipitated an extensive search for equivalent responses in vitro. A possible explanation for the difficulty in demonstrating post-junctional α_2-adrenoceptors in vitro is due to lack of humoral agents normally present in the whole animal. We have shown previously in the perfused rat tail that after raising the tone with vasopressin, responses to UK14304 were prazosin-resistant, rauwolscine-sensitive(at basal tone UK14304 did not produce a contraction) (Templeton et al., 1989). In this study we have examined the response of the perfused rabbit ear to UK14304 to determine the extent of α_2-adrenoceptor involvement in mediating pressor responses.

New Zealand White rabbits (2-3kg) were killed by stunning followed by exsanguination. The ear was removed and the ear artery cannulated and perfused at a flow rate of 2-3ml/min with a modified Krebs-Henseleit solution (29°C) containing 2% Ficoll. Basal perfusion pressure stabilised between 30-70mmHg and responses to bolus injections of increasing doses of the agonists(10µl) were measured. Where appropriate antagonists were included in the perfusate for 45 minutes before repeating the agonist dose response curve. In some ears tone was raised with U46619(TxA_2 mimetic) to approximately 30-50mmHg above baseline and the agonists were studied again in the same way. Results are expressed as the ED_{10} at basal tone (this is the -log of the dose of UK14304 required to increase perfusion pressure by 10mmHg which was approximately half of the control maximum response obtained) and ED_{30} at elevated tone(as before but tone raised by 30mmHg, which was approximately half of the control maximum response obtained to enable comparison with basal tone) along with the maximum response. UK14304 produces dose-dependent increases in perfusion pressure at basal and elevated tone, with results in Table 1. In the presence of rauwolscine a true maximum was not attained values represent the highest concentration possible.

Table 1

	Basal tone				Elevated tone with U46619			
	Control	+prazosin	Control	+rauwolscine	Control	+prazosin	Control	+rauwolscine
Max (mmHg)	20.2±3	20.9±3	17±2	11±2	57±10	72±10	63±10	39±3
$ED_{10/30}$	10.05±0.3	9.77±0.17	10.02±0.28	8.85±0.2*	9.95±0.17	10.3±0.16	10.01±0.36	9.01±0.2*

Values expressed as mean ± sem *0.05<P<0.01 indicates significantly different from corresponding controls

Responses to bolus doses of both NA and Phenylephrine , regardless of basal or elevated tone, could be inhibited by prazosin.
These results suggest a mixed population of α_1 and α_2-adrenoceptors mediating pressor responses in the rabbit ear. In contrast to our results with the perfused rat tail α_2-adrenoceptor expression is not dependent on pre-existing tone.

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110P MODULATION OF POST-JUNCTIONAL α_2-ADRENOCEPTORS IN THE RABBIT DISTAL SAPHENOUS ARTERY

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In the rabbit distal saphenous artery, noradrenaline (NA), under standard in vitro conditions, evokes a contraction which is wholly sensitive to prazosin, insensitive to rauwolscine and accordingly mediated solely by α_1-adrenoceptors (McGrath et al., 1989). If however, blockade of α-adrenoceptors is effected, under conditions designed to protect α_2-adrenoceptors, a small residual contraction to noradrenaline (NA) is observed. This response is greatly enhanced in the presence of angiotensin II (AII) and the enhanced portion of the response is abolished, either by rauwolscine, or by the AII-receptor antagonist saralasin (Dunn et al., 1989), suggesting an enabling role for AII in the demonstration of post-junctional α_2-adrenoceptors in the rabbit saphenous artery. This present study examined the possible role of other excitatory ligands in unmasking post-junctional α_2-adrenoceptors. Possible mechanisms for interactions between agonists are discussed.
Male New Zealand White rabbits (1.5-2.5Kg) were killed by stunning followed by exsanguination. Lengths of distal saphenous artery (2-4mm) were suspended under 1g tension for isometric recording in a modified Krebs solution (for composition see Daly et al., 1988), containing propranolol (1µM) and cocaine (10µM). After addition of rauwolscine (1µM) and saralasin (0.3µM), to prevent binding to α_2- and AII-receptors respectively, tissues were incubated in phenoxybenzamine (0.1 - 0.3µM for 25 min) to irreversibly block α_1-adrenoceptors. Control concentration-response curves (CRC's) were constructed to NA (10^{-10}-10^{-4}M, mean PD_2 (-log concentration of the EC50); 6.72±0.1) and results expressed as % of maximum control response. NA CRC's were then repeated in the presence of various agonists, using doses of agonist producing 25-30% of maximum response.
In the presence of AII (0.1µM) or of neuropeptide Y (NPY, 30nM), NA-evoked responses were significantly enhanced (mean PD_2; 7.86±0.26 and 9.27±0.37 respectively, $p<0.001$), giving approximately 10 and 100-fold leftward shifts respectively, with increased maximum contraction. In contrast, neither endothelin 1, nor 5-hydroxytryptamine, (each1nM) significantly altered the response to NA after 'receptor protection'. The enhancement of NA responses evoked by AII and by NPY was mimicked by the phorbol ester phorbol 12-myristate 13-acetate (PMA, mean PD_2; 8.79±0.18, $p<0.001$), but not by KCl (30mM). Accordingly, while AII is not the only ligand capable of enhancing post-junctional α_2-adrenoceptors, not all agonists capable of evoking contraction in the rabbit saphenous artery show this ability. The absence of any effect of KCl suggests that elevation of $[Ca^{2+}]_i$ does not alone account for the enhancing effects of AII and NPY on NA-evoked responses, while the ability of PMA to mimic these effects suggests a possible involvement of protein kinase C in this response.

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111P PERFUSED OVINE RENAL GLOMERULAR VASCULATURE: A MODEL TO STUDY THE NATURE OF STRUCTURAL, MYOFIBROBLAST CONTRACTILITY

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The vasculature of the mammalian kidney, both extra and intrarenal is complex in terms of both its structural organisation (Graves, 1954) and its in vitro reactivity (Nizet, 1975). The usual in vitro model is the perfused rat kidney, achieved via cannulation of the dorsal aorta or renal artery. These cannulation techniques make it impossible to differentiate an extrarenal effect from those contributed by the intrarenal vessels and glomeruli.

It has been found that cannulation of intrarenal vessels of the rat is technically difficult and unreliable and so an alternative preparation was sought to characterise the nature of intrarenal and glomerular reactivity. For this purpose the ovine kidney was chosen. Ovine kidneys (45-120g) were obtained from a local abattoir and used within 30 minutes. Regional perfusion of the kidney was achieved by selective cannulation of the interlobar and arcuate arteries. Perfusion pressure was measured in the inlet cannula using a Bell and Howell (type 4-422) pressure transducer. Control experiments showed that the tissues were stable and sensitive for a period of >4hr. Drugs used were NA(2-4nmol), 5-HT (2-4nmol), KCl (0.8-3.2mmol), angiotensin (0.1-0.4μg), tetracaine (4-16mg) and the H_1 antagonists mepyramine (4-16mg), methapyrilene (4-16mg), diphenhydramine (4-16mg) either administered as bolus doses or infusions. All the responses were found to be dependent on both the site of arterial cannulation and the type of injection method used. For example, when bolus doses of mepyramine were used, shown elsewhere to contract myofibroblasts (Cross & Naylor, 1989), there was no response. In contrast, when mepyramine (4-16mg) was infused there was a significant ($P<0.05$) increase of perfusion pressure (7.1-11.06mm Hg). The rank order of effectiveness of the agonists used was: 5-HT > NA > KCl > angiotensin II > tetracaine > mepyramine > methapyrilene > diphenhydramine. To establish the area which produced the responsiveness of the extrarenal vessel in selected experiments, the supply to the glomerular region via the interlobar artery was sectioned. The responses to KCl were unaffected, contrasting with the abolition of the response to mepyramine. These preliminary results suggest that glomerular structures may contain a mepyramine sensitive contractile cell. A candidate for such a cell type is the myofibroblast which has an unusual range of pharmacological sensitives and has structural similarities to the mesangial cell which has been described in the literature (Nagle et al, 1975).

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112P THE RELAXANT EFFECTS OF ATRIAL NATRIURETIC PEPTIDE AND NITROPRUSSIDE ON PULMONARY ARTERIES FROM RATS WITH MONOCROTALINE-INDUCED PULMONARY HYPERTENSION

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Atrial natriuretic peptide (ANP) is a potent relaxant of isolated pulmonary resistance vessels (PRV) from rats with hypoxic pulmonary hypertension (PH; Rogers & Morice, 1990). In this study ANP has been examined on PRV from rats with PH induced by monocrotaline (MCT). ANP has been compared with nitroprusside (NP) since, in isolated main pulmonary arteries from MCT-treated rats, the potency of NP was less than in controls (Wanstall & O'Donnell, 1990).

Wistar rats treated with MCT (105 mg/kg s.c. 4 weeks before the experiment) had right ventricular hypertrophy indicating PH (R. ventricle/[L. ventricle+septum], MCT 0.60±0.08, n=6; saline-treated controls 0.28±0.02, n=6, $P<0.01$). PRV were isolated and mounted on a small vessel myograph (Cambustion Biological, Cambridge) at resting forces equivalent to transmural pressures of 35 (MCT) or 17.5 (control) mmHg. Preparations were submaximally contracted with $PGF_{2\alpha}$ (20 µM). Cumulative concentration-response (relaxation) curves to ANP and NP were obtained, followed by a maximal relaxant response to pinacidil (PIN, 100 µM).

In PRV from MCT-treated rats the potency (neg log EC50) of ANP was not different from controls, but that of NP was reduced (* $P<0.05$; Table 1). Maximum relaxation to ANP and NP (a) was below the pre-$PGF_{2\alpha}$ baseline (i.e. >100%, indicating inherent resting tone) in MCT PRV, but not in controls, and (b) was less than that to PIN in all vessels.

Table 1

	ANP		NP	
	CONTROL	MCT	CONTROL	MCT
neg log EC50	8.55 ± 0.11 (4)	8.44 ± 0.18 (4)	7.63 ± 0.11 (4)	7.13 ± 0.17 (4)*
Max. relaxation (% reversal of $PGF_{2\alpha}$)	76 ± 6.6 (4)	131 ± 25.8 (4)	81 ± 2.9 (4)	126 ± 11.1 (4)
PIN relaxation (% reversal of $PGF_{2\alpha}$)	112 ± 8.8 (4)	204 ± 39.1 (4)	102 ± 2.9 (4)	158 ± 18.5 (4)

The increase in maximum relaxation to ANP, with no loss in potency, in PRV from rats with MCT PH agreed with previous data from rats with hypoxic PH (Rogers & Morice, 1990). These findings, together with reports that circulating levels of ANP are increased in PH (Morice *et al.*, 1990), are compatible with the hypothesis that ANP could act as a modulator of pulmonary vascular resistance, especially in PH.

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113P LACK OF EFFECT OF LONG-TERM STREPTOZOTOCIN DIABETES ON VASCULAR RESPONSIVENESS OF THE RAT MESENTERIC BED *EX VIVO*

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Endothelial dysfunction has been found in diabetes but the effect of diabetes on responsiveness to endothelium dependent vasodilators is unclear. Reports have variously shown a reduction (Durante et al. 1988), or an increase (Bhardwaj & Moore, 1988) in responsiveness to acetylcholine of arteries removed from diabetic rats. The aim of the present work was to determine the effect of chronic experimental diabetes on the responsiveness of the rat isolated mesenteric bed to noradrenaline (NA) and to endothelium-dependent vasodilators (acetylcholine (Ach), adenosine triphosphate (ATP)) and endothelium-independent vasodilators (sodium nitroprusside (SNP)).

Mesenteric beds were removed from rats 15-17 weeks after injection of streptozotocin (STZ, 35mg/kg i.v.) or vehicle and perfused (2ml/min) with Krebs buffer (37°C; 95% O_2/5% CO_2). A blood sample for glucose determination was obtained at the end of the surgery. After determining cumulative dose-response curves to NA, preparations were perfused with normal Krebs for 15 min. Cumulative dose-response curves to Ach, ATP and SNP were then determined after increasing the perfusion pressure with NA (10^{-5}M) with 15 min perfusion with normal Krebs between each curve. STZ-treated rats showed polydypsia, polyuria and glycosuria with marked hyperglycaemia (plasma glucose, mmol/l, 39±2 vs 15.7±1.7, $P<0.01$) and reduced body weight (353±11.3 vs 523±16g, $P<0.01$). All STZ-treated rats had cataracts by 15 weeks.

NA (10^{-7}-10^{-4}M) produced concentration-dependent increases in perfusion pressure. There was no significant difference between responses obtained in control preparations and those obtained in preparations from STZ-rats (EC_{max50}, control 1.1±0.34 x 10^{-5}M vs 0.89±0.25 x 10^{-5}M). Ach, ATP and SNP (10^{-7}-2.6 x 10^{-5}M) produced concentration dependent vasodilator responses. The responses to Ach or to ATP were markedly depressed by perfusing for 30s with distilled water containing 0.1% Triton X100 to disrupt the endothelium. This procedure increased responsiveness to NA by about two-fold but did not modify responses to SNP. STZ treatment did not modify responsiveness to Ach, ATP or SNP. For example, responses to 1.6 x 10^{-6}M Ach, ATP or SNP, expressed as a % relaxation of the noradrenaline-elevated perfusion pressure were, respectively, 72.8±11.5, 35.5±7.2 and 60.1±7.8 in preparations from STZ-treated rats compared with corresponding values in control preparations of 73.7+8.2, 39±6.3 and 72.1±6.

Thus chronic STZ-induced diabetes in rats does not modify *ex vivo* responsiveness to NA. Moreover neither cholinoceptor- nor P_{2Y}-purinoceptor-mediated endothelial dependent vasodilator responses are altered. The reasons for the discrepancies in the literature remain unexplained. It is possible that the dysfunction is evident only in the continued presence of the diabetic environment, which is absent in *ex vivo* experiments.

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114P COMPARISON OF THE EFFECTS OF HYPOXIA AND CONTRACTILE AGONISTS ON HUMAN ISOLATED PULMONARY ARTERY

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Hypoxia causes pulmonary hypertension and contraction of isolated pulmonary artery rings. The aim of this study was to investigate the effects of hypoxia on human pulmonary artery rings and to compare the responses produced with various contractile agents.

Lung segments were obtained from 15 patients (mean age 62±2, range 44-76) undergoing thoracic surgery. Pulmonary arteries of large (2.2-4.5 mm) and small (0.38-0.64 mm) diamater were cut into rings and suspended in Krebs-Henseleit solution (37°C) under optimum resting force of 1.6 g and 0.4 g respectively. Isometric force generated per cross sectional area of the vessel was measured. The solution was aerated with 20% O_2-5% CO_2 (normoxic) and 95% N_2-5% CO_2 (hypoxic) to yield PO_2 levels in the bath of 139±1 and 4±0.5 mmHg respectively.

Under optimal resting force, introduction of hypoxia caused a contraction both in large (28.8±18.0 $g.cm^{-2}$, n=5) and small (7.6±5.4 $g.cm^{-2}$, n=5) artery rings. In precontracted (endothelin, U46619 or 5-HT) large and small pulmonary artery rings, hypoxia caused a further contraction. Cumulative concentration-response curves were constructed to the contractile agonists shown in Table 1. Both large and small pulmonary artery rings responded to endothelin and KCl. In small pulmonary artery rings, 5-HT gave a small contraction only, but U46619 produced a contraction with a similar maximum to that of endothelin. These preparations were weakly responsive to histamine (19 ± 3 µM, n=3) and unresponsive to phenylephrine (0.1 µM - 0.1 mM, n=3).

Table 1

	Large Pulmonary Artery		**Small Pulmonary Artery**	
	EC_{50}	Maximum Contraction ($g.cm^{-2}$)	EC_{50}	Maximum Contraction ($g.cm^{-2}$)
Endothelin	7.9 ± 3.0 nM	33 ± 14	7.6 ± 2.9 nM	50 ± 2
5-HT	467 ± 126 nM	49 ± 21	290 ± 160 nM	10 ± 4
U-46619	-	-	27 ± 2.5 nM	56 ± 12
KCl	31 ± 2.8 mM	108 ± 31	40 ± 6.3 mM	148 ± 14

All values are given as mean ± s.e.m., n=3-6.

These studies demonstrated that hypoxia produces a contraction of human isolated pulmonary artery rings both under baseline tension and when precontracted. In the large diameter rings, the contraction produced by hypoxia was comparable in size to the maximum contraction produced by endothelin or 5-HT.

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115P METABOLISM OF AMINOACETONE BY SEMICARBAZIDE-SENSITIVE AMINE OXIDASE ACTIVITY IN HUMAN UMBILICAL ARTERY

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Aminoacetone (AA) is produced by mitochondrial metabolism of threonine and glycine in animal cells, and it has been found in urine from man and other species. Although it has been shown that AA is converted to methylglyoxal (MG) by a soluble plasma amine oxidase in ruminants (Ray & Ray, 1983), the possible role of tissue amine oxidases in metabolizing AA in various species has not been studied extensively. Vascular smooth muscle cells of human blood vessels, including umbilical artery, have been shown to possess a semicarbazide-sensitive amine oxidase (SSAO) which metabolizes the synthetic amine benzylamine (BZ) (Precious & Lyles, 1988). AA has been synthesized in our laboratory and then studied as a potential substrate for this enzyme.

Kinetic studies, using Lineweaver-Burk analysis determined the influence of AA concentrations (50 - 200 μM) upon the metabolism of [^{14}C]-BZ (0.1 - 1 mM), an established substrate for SSAO in low-speed supernatants (600g/10 min) from human umbilical artery homogenates. The metabolism of BZ (Km = 283 ± 19 μM) was inhibited competitively by AA (Ki = 83 ± 9 μM) (mean values ± s.e. from five tissues). Incubation of AA alone with these supernatants produced MG which was detected by the absorbance at 550nm of its 2,4-dinitrophenylhydrazine adduct. However, incubation of exogenously-added MG with these fractions resulted in a time-dependent disappearance of MG, probably indicating the presence of the (predominantly) cytosolic MG-metabolizing glyoxalase enzyme system. This loss did not occur when MG was incubated with membrane fractions prepared by centrifugation (105,000 g/1h) of the supernatants above, and so the latter were used subsequently for estimation of AA metabolism (25 - 125μM), yielding apparent kinetic constants of Km: 93 ± 23 μM, Vmax = 270 ± 49 nmoles MG/h/mg protein (n = 7). Higher concentrations of AA produced "apparent" high-substrate inhibition. Metabolism of 100 μM AA was inhibited completely in membranes preincubated with 1mM propargylamine, an inhibitor of SSAO as well as monoamine oxidase (MAO) activities, but was not affected by the MAO-selective inhibitor pargyline (1mM).

The relative lack of activity and high Km of human SSAO activity towards many endogenous monoamines previously examined has prevented a function for this enzyme in amine metabolism in human tissues from being established. The present results indicate that AA is an in vitro substrate with a much lower Km than other possible physiological substrates studied. The possibility that AA deamination by SSAO in vivo may be a source of physiologically and/or toxicologically active MG and its metabolite lactoylglutathione (produced via the glyoxalase pathway) (Thornalley, 1990) may be worthy of consideration.

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116P RESISTANCE OF CHOLINERGIC VASODILATATION TO ISCHAEMIA OF THE RAT MESENTERIC VASCULAR BED *IN VIVO* AND *IN VITRO*

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Vasodilatation in response to acetylcholine (ACh) is mediated by the release of a relaxing factor from the endothelium (Furchgott & Zawadzki, 1980). Vascular endothelial cells are sensitive to ischaemia; for example, it has been reported that occlusion of the superior mesenteric artery (SMA) for 30 min impairs the ability of ACh to attenuate vasoconstrictor responses to noradrenaline (NA) in the rat mesenteric arterial bed (MAB) *in vivo* (Altura, 1985; Whalley & Oldham, 1988). However, both these studies used bolus co-administration of ACh and NA. We have re-examined this phenomenon in the rat MAB, *in vivo* and *in vitro* .

Male Sprague-Dawley rats (200-250g) were anaesthetized with pentobarbitone (60 mg/kg, i.p.). The *in vivo*, blood-perfused mesentery (Jackson & Campbell, 1980) was prepared by cannulating the aorta to supply blood to the SMA, via a peristaltic pump (2 ml/min). Blood pressure was measured both distal and proximal to the pump. After a 30 min recovery period, pressure in the MAB was elevated by infusion of phenylephrine (45 μg/min), and carbachol (1ng-1μg) was injected in 1 μl boluses. Blood flow was arrested for 1h and the carbachol tests repeated. The *in vitro* rat MAB (McGregor, 1965) was prepared by perfusing the MAB with oxygenated Krebs-Bicarbonate solution at 37°C (2 ml/min), via the SMA. After ligating the major branches of the SMA, the mesentery was trimmed away from the gut and placed on a perforated platform in an organ chamber at 37°C. The preparation was also superfused with Krebs solution at 37°C. Perfusion pressure was measured distal to the pump. After a 30 min equilibration period, pressure was elevated by perfusion with phenylephrine (2 μg/ml). 1 μl boluses of carbachol (0.3 ng-1 μg) were injected at 5 min intervals. The pump was switched off for 1h, and the tests repeated. Alternatively, following the initial 30 min equilibration period the flow was stopped for 1 or 2h before testing with carbachol.

In vivo: Carbachol (1ng-0.1μg) elicited dose-related vasodilator responses; a higher dose (0.3μg) had less effect. The ED_{25} was 12 ng (n = 5). Following 1h ischaemia, the responses were unchanged. *In vitro:* Carbachol (1ng-0.1μg) elicited dose-related vasodilator responses; a higher dose (0.3 μg) had less effect. The ED_{25} was 3.2 ng (n = 5). Following 1h ischaemia, responses to carbachol were reduced (ED_{25} = 180 ng; $P < 0.05$). However, this also occurred in a group of time-matched controls (ED_{25} = 100 ng; n = 5; $P < 0.05$). When tests were done after 1h or 2h ischaemia without pre-ischaemia injections of carbachol, the responses were the same as time-matched controls.

In summary, 1h of ischaemia *in vivo* did not impair vasodilator responses to carbachol in the rat MAB. *In vitro*, there was desensitization of the preparation 1h after the initial carbachol dose-response curve, but there was no additive effect of ischaemia. Also, after either 1 or 2h of ischaemia the carbachol dose-response curves were the same as time-matched controls. The effects of ischaemia *in vitro* in this preparation differ from those reported by Zambetis & Grossman (1988), who found impairment of responses to ACh after 2h, but not 1h, of ischaemia.

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117P RELATIONSHIP BETWEEN ENDOTHELIAL CELL ACTIVATION AND SUBSEQUENT NO SYNTHASE ACTIVITY: INHIBITION BY OXYGEN DERIVED FREE RADICALS

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Endothelial cells release a relaxing factor (EDRF) which has been identified as nitric oxide (NO; Palmer et al., 1987) or a related molecule (Myers et al., 1990). The enzyme system in endothelial cells responsible for EDRF formation (NO synthase) is dependent on NADPH, activated by Ca2+ and located mainly in the particulate fraction of cultured cells (Förstermann et al., 1991) and solely in the particulate fraction of native cells (Mitchell et al., 1991). Endothelial cells can release EDRF in a sustained manner if continuously activated (Mitchell et al., 1990). Under these conditions it may be expected that other free radicals are also produced. The purpose of this study was to assess the effect of prior exposure of intact endothelial cells to Ca2+ and Ca2+ ionophore A23187, on subsequent NO synthase activity. In addition, we incubated semi-purified NO synthase with NO, 3-morpholinosydnomine (SIN 1) and pyrogallol to test the effects of NO and superoxide generation on enzyme activity (measured in terms of L-citrulline production from L-arginine).

Cultured bovine aortic endothelial cells (BAE) were incubated for 30 min either in Ca2+ free Krebs' solution, regular Krebs' solution or Krebs' solution + A23187. The BAE were then homogenized and soluble and particulate fractions prepared (Förstermann et al., 1991). The NO synthase activity of each fraction was measured by cGMP accumulation (t= 3min) in rat fetal lung fibroblasts (RFL-6 cells; Ishii et al., 1989). Particulate fractions (30 μg/ml protein) prepared from BAE exposed to Ca2+ free Krebs' solution increased cGMP levels in RFL-6 cells to 91.7 ± 10 pmol/ 10^6 cells in the presence of superoxide dismutase (SOD; 10 U/ml), NADPH (100 μM) and L-arginine (100 μM) (n=6). However, BAE exposed to Ca2+ yielded lower particulate NO synthase activity (55 ± 10 pmol cGMP; n=6). The particulate activity was even lower from BAE exposed to Ca2+ and A23187 (1 μM; 30 ± 9 pmol cGMP; n=6). In contrast the cytosolic NO synthase activity was unaffected following exposure of whole cells to Ca2+ and A23187 (n=3). In separate experiments particulate NO synthase was solubilized according to Förstermann et al., (1991) and the activity measured by ^{3}H L-citrulline formation from ^{3}H L-arginine (t= 20 min; at 25 °C). SIN 1 (1-100 μM), pyrogallol (0.1-100 μM) or NO (1-100 μM) inhibited citrulline formation in a dose dependent manner (n=3-6).

These results show that Ca2+-induced activation of endothelial cells causes a profound inhibition in the particulate NO synthase activity without affecting soluble NO synthase, possibly by generation of NO and/or superoxide. Free radical induced inhibition of NO synthase could be important during pathophysiological conditions such as ischemia and subsequent reperfusion.

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118P SELECTIVE PURINERGIC REGULATION OF NITRIC OXIDE RELEASE

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The overall physiopharmacological significance of the recently discovered potent vasodilator nitric oxide (NO) (Palmer et al., 1987) on the cardiovascular system has still to be fully evaluated. The mechanisms by which the liver, for example, is able to accommodate reductions in portal vein (PV) flow by compensatory alterations in hepatic arterial (HA) flow, in an attempt to retain the original total hepatic blood flow, remain poorly understood. We have recently shown using a novel dual-perfused isolated rabbit liver preparation (Alexander et al 1991) that the rabbit HA vascular bed has a distinct population of P_1 (A_2)- and P_2-purinoceptors (Mathie et al 1991a) and that ATP may elicit the release of NO via activation of endothelial P_{2y}-purinoceptors (Mathie et al 1991b, Ralevic et al 1991).

The livers of 10 New Zealand White rabbits were perfused simultaneously through the HA and PV at fixed flow rates of 23 ± 1 and 77 ± 1 ml min^{-1} 100g $liver^{-1}$ (mean ± s.e.) respectively, which correspond to perfusion pressures of 134 ± 13 mmHg and 10 ± 2 mm Hg. The tone of the preparation was raised by the inclusion of noradrenaline (10^{-5}M) in the perfusate. Dose-dependent HA vasodilatations, measured as a transient decrease in perfusion pressure, were obtained to adenosine, ATP and the acknowledged endothelium-dependent vasodilator acetylcholine (ACh): dose-response curves were constructed prior to the addition of the NO synthase inhibitors N-monomethyl-L-arginine (L-NMMA, n=6) and N-nitro-L-arginine methyl ester (L-NAME, n=4) at 30 and 100 μM concentrations for each compound. L-NMMA (30μM) did not alter the pD_2 of ACh (8.7 ± 0.1) or ATP (8.4 ± 0.3): however, at 100 μM, L-NMMA significantly attenuated responses at the three highest doses of ACh and ATP but this did not alter the control pD_2 values. L-NAME (30μM) significantly attenuated responses to ACh, reducing the pD_2 from 9.0 ± 0.2 to 8.6 ± 0.2. L-NAME also significantly inhibited responses to ATP at 30μM reducing the pD2 from 8.4 ± 0.3 to 7.6 ± 0.2 (Student's unpaired t-test) thus confirming L-NAME to be the more potent of the two inhibitors. However, the 100μM concentration of L-NAME did not reduce the pD_2 further to either ACh or ATP. Dose-response curves to adenosine were not significantly altered by the NO synthase inhibitors but the specific P_1-purinoceptor antagonist 8-phenyltheophylline (10μM) significantly attenuated adenosine mediated vasodilatations thus confirming these were not due to NO release and/or stimulation of NO synthase. Patency of the preparation was confirmed by the absence of any inhibition of vasodilatation produced by the direct smooth muscle vasodilator sodium nitroprusside (SNP); indeed a potentiation of the responses to SNP by both L-NMMA and L-NAME was observed. It is concluded that the release of NO in the rabbit HA vascular bed is at least in part the result of activation of P_{2y}-purinoceptors by ATP.

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119P THE EFFECT OF EXTRACELLULAR [Ca^{2+}] REDUCTION, NIFEDIPINE, RYANODINE AND L-NAME ON *IN VITRO* HYPOXIC PULMONARY VASOCONSTRICTION IN THE RABBIT

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Hypoxic pulmonary vasoconstriction (HPV) is thought to involve increases in both extracellular and intracellular [Ca^{2+}] (Hoshino *et al.*, 1988) and be modulated by EDRF *in vivo* (Persson *et al.*, 1990). Here, this is further investigated in rabbit pulmonary arteries *in vitro*. Isolated vascular rings (3-5mm) were taken from the main pulmonary artery and its first, left, branch from NZW rabbits (2-3 kg), newly killed by stunning and cervical dislocation. Rings were suspended in organ baths containing Krebs saline at 37°C under 2g force and gassed with 5% O_2, 6% CO_2 balance N_2 (bath O_2 tension=45.2±0.5mmHg, CO_2=35.7±0.4mmHg, pH=7.39±0.03, n=6) until challenged (5 min) with hypoxia and gassed with 0% O_2, 6% CO_2 balance N_2 (bath O_2 tension=5.6±0.6mmHg, CO_2=35.8±0.5mmHg, pH=7.37±0.01, n=6). Changes were evaluated with a paired Student's *t* test where n>4.The presence of an agonist was required to initiate consistent HPV (shown here with both noradrenaline (NA) and endothelin-1) which was dependent on the presence of the vascular endothelium. HPV amplitude increased as vascular tone increased i.e. increasing tone in the main artery branch with $3x10^{-8}$M NA (+200±52 mg tension g wt) induced an HPV of 132±62 mg and increasing tone with $3x10^{-6}$M NA (+2100±319.9 mg) induced an HPV of 746±136 mg (all n=6, $P<0.05$). These were initially transient (1-2 min) and followed by pulmonary relaxation, but, after successive exposures to NA and hypoxia, they lasted the duration of hypoxic exposure and were increased in amplitude. HPV were not initiated by raising vascular tone with KCl (50-100mM) and 10^{-5}M nifedipine did not significantly reduce HPV at any of the 6 doses of NA used in either the main artery or its branch. e.g. in the main artery, in the presence of $3x10^{-7}$ NA, HPV was 290±79 mg before, and 196±37.6 mg 45 min after 10^{-5}M nifedipine (n=5,NS). After equilibration in Ca^{2+} free Krebs (plus 0.05mM EGTA) the response of the main artery to $3x10^{-7}$ NA was reduced from 1306±345 mg to 652±185 mg (n=4) and further reduced to 344±253 mg after 45 min exposure to 10^{-5}M ryanodine (an inhibitor of intracellular Ca^{2+} release). Likewise, HPV was reduced from 252±91 mg to 85±37 mg tension in Ca^{2+} free Krebs (n=4) and further reduced to 12.5±12.5 mg after exposure to ryanodine.

10^{-4}M N^{ω}-nitro-L-arginine methylester (L-NAME, an inhibitor of nitric oxide (NO) synthetase) increased tone in both the main pulmonary artery and its branch. This effect was significantly greater in the branch where it increased tone by 1071±150 mg compared with an increase of 533±65 mg in the main artery (both n=6, $P<0.01$). In the pulmonary branch, despite the increase in tone caused by L-NAME (in the presence of $3x10^{-6}$ NA), L-NAME reduced HPV from 1183±137 mg to 488±120 mg (n=6, $P<0.01$) and was increased again after exposure to $2x10^{-4}$ L-arginine to 921±156 mg ($P<0.01$). In the main artery L-NAME reduced HPV from 1146±224 mg to 977±202 mg (n=6, $P<0.01$) but this effect was not reversed by $2x10^{-4}$ L-arginine.

In conclusion, *in vitro* HPV in the rabbit depends on agonist-induced increases in both intra- and extracellular Ca^{2+} mobilization. Activation of voltage operated Ca^{2+} channels alone does not facilitate HPV. Endogenous NO has less influence on vascular tone in the main pulmonary artery than in its smaller branch and *in vitro* HPV may involve inhibition of NO production. Successive exposure to hypoxia and NA prolongs HPV similar to that normally observed *in vivo*.

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120P DOES CAPTOPRIL VASODILATE BY MECHANISMS OTHER THAN BY ACE INHIBITION?

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Captopril was the first angiotensin converting enzyme (ACE) inhibitor to be introduced for clinical use. It differs from most other ACE inhibitors in that it contains a thiol group, which has been suggested to enhance the action of endothelium derived relaxing factor (EDRF) (Vanhoutte *et al.*, 1989), either by scavenging free radicals, which accelerate inactivation of EDRF, or perhaps by forming a more stable and possibly more potent, nitrosothiol adduct. More recently it has been suggested that captopril may also be a calcium channel blocker (Jeremy *et al.*, 1990, Bryant *et al.*, 1991). Our experiments were designed to investigate whether therapeutic concentrations of captopril either blocked voltage dependent calcium channels or enhanced the action of EDRF in resistance vessels *in vitro*.

Rat mesenteric (diameter 193-308μm, n=8) or human subcutaneous resistance arteries (diameter 132-448μm, n=7) were mounted in a small vessel myograph, bathed in physiological salt solution, bubbled with 95% O_2/ 5% CO_2 and maintained at 37°C. Vessels were contracted by depolarisation using 118mM K^+ solution (4 rat, 4 human) or 10μM noradrenaline (4 rat, 3 human) and captopril (10nM-100μM) was added once tone was stable. In the second series of experiments rat vessels (diameter 162-308μM, n=8) were preconstricted with 10μM noradrenaline and acetylcholine (1nM-1μM) was added cumulatively. The protocol was repeated in the presence of 1μM captopril after a 30 minute recovery period. A third concentration-response curve was constructed to acetylcholine alone after a further recovery period. Relaxation is expressed as % reduction of precontracted tone and concentration-response data were analysed by fitting to a logistic function by non-linear regression. pD_2 values and maximum responses (Emax) are given as means ± s.e.means of n observations.

Captopril did not relax rat or human vessels precontracted by either depolarisation or noradrenaline. Nor did captopril enhance the action of acetylcholine; the three consecutive acetylcholine dose-response curves had pD_2s and Emaxs of 7.45±0.14 and 85±6% (first control), 7.59±0.08 and 84±3% (with captopril) and 7.50±0.13 and 83±5% (second control). There was no significant difference between these curves.

In conclusion captopril does not relax isolated resistance arteries from rat or man. This is not consistent with the proposed inhibitory effect of captopril on voltage dependent or receptor operated calcium channels. Furthermore captopril does not enhance endothelium-dependent relaxation in these experimental models of resistance arteries.

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121P EFFECTS OF REPEAT ORAL ADMINISTRATION OF THE POTASSIUM CHANNEL ACTIVATOR, BRL 38227, IN SPONTANEOUSLY HYPERTENSIVE RATS

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BRL 38227 lowers blood pressure (BP) in animal models of hypertension (Clapham et al, 1991). However, to date there has been only one report, using Dahl Salt-sensitive rats, about the effects of repeat oral administration of BRL 38227, 0.5 mg.kg^{-1} (Hirawa, et al 1989). In this investigation, the effects of daily oral dosing of BRL 38227, for up to 8 weeks, on BP, plasma renin activity (PRA), aldosterone (ALD), parent drug levels, plasma electrolytes, packed cell volume (PCV) and heart weight/body weight ratio in conscious SHR have been examined.

Male SHR (12 weeks of age at start of study) were divided into 3 groups of 24 animals receiving vehicle (2.5 ml.kg^{-1}), BRL 38227 (0.05 mg.kg^{-1}) or BRL 38227 (0.1 mg.kg^{-1}) daily by oral gavage. After 1, 2, 4 or 8 weeks of dosing, six rats were removed from each group and systolic BP was recorded indirectly via a tail-cuff method (W&W recorder), predose and 1.5, 3 and 6h after drug administration. The following day, the same rats were anaesthetised with pentobarbitone sodium (60 mg.kg^{-1} i.p.) 1.5h after drug treatment. Following laparotomy the renal blood vessels were clamped and whole blood collected into heparinized syringes to obtain plasma for PRA and ALD determinations (using commercially available RIA kits), electrolytes, (using a Kone ion selective analyser) and PCV (using an haematocrit centrifuge). Plasma levels of parent drug were assayed by an HPLC technique using fluorescence detection (Beerahee et al, 1991). The heart was removed, the left ventricular wall and septum were dissected and stored in 10% formalin prior to weighing.

BRL 38227 (0.05 mg.kg^{-1}day^{-1}) was a threshold antihypertensive dose in SHR, a significant ($p<0.05$; Dunnett's) effect occurring 1.5h post-dose only at the two week study period. At a dose of 0.1 mg.kg^{-1}day^{-1}, BRL 38227 produced consistent and significant ($p<0.05$) falls in systolic BP for up to 8 weeks of dosing. Daily oral dosing of BRL 38227, 0.05 and 0.1 mg.kg^{-1} yielded, 1.5h post-dose, levels of parent drug in plasma of 3-7 and 10-12 ng.ml^{-1} respectively with no evidence of accumulation. PRA was significantly ($p<0.05$) raised by 0.1 mg.kg^{-1}day^{-1} BRL 38227, but ALD levels were not significantly different from the vehicle group. There were no changes between groups in plasma Na^+ and K^+, PCV or relative heart weight after 8 weeks.

In conclusion, daily oral administration of BRL 38227 for up to 8 weeks produces reproducible anti-hypertensive responses in SHR without evidence of drug accumulation in plasma. Consistent with its mode of action as a vasorelaxant, increases in PRA were observed. Despite this implied increase in sympathetic activity. changes in cardiac weight (relative to body weight) were not observed. Plasma electrolyte, PCV and body weight determinations suggest that fluid retention may not occur following long-term administration of BRL 38227 in man.

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122P CARDIAC HAEMODYNAMIC EFFECTS OF L-ARGINYL-L-PHENYLALANINE IN CONSCIOUS RATS

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The dipeptide L-arginyl-L-phenylalanine (Arg-Phe) which is part of the amino acid sequence of the molluscan neuropeptide, FMRF-amide, has recently been reported to elicit pressor and tachycardic responses in anaesthetized rats (Thiemermann *et al.*, 1991). Now we have measured the cardiac haemodynamic effects of Arg-Phe in conscious rats.

Male, Long Evans rats (n=6, 350-450 g) were anaesthetized with sodium methohexitone (60 mg kg^{-1} i.p.) and an electromagnetic flow probe (Skalar, Delft) was implanted around the ascending aorta via a transthoracic approach. At least 7 days later, the animals were re-anaesthetized (as above) and had intravascular catheters implanted. One to 2 days later, each animal received Arg-Phe (50 and 100 μg kg^{-1} i.v.) before and during the sequential infusion of the β_2-adrenoceptor antagonist, ICI 118551 (0.2 mg kg^{-1}; 0.1 mg kg^{-1} h^{-1}), ICI 118551 plus phentolamine (1 mg kg^{-1}; 1 mg kg^{-1} h^{-1}) and ICI 118551 plus phentolamine plus atenolol (1 mg kg^{-1}).

Arg-Phe (50 and 100 μg kg^{-1}) evoked rapid pressor responses (38 ± 5 and 51 ± 2 mmHg, respectively; mean ± s. e. mean; $P<0.05$, Student's t test), as previously reported. However, there was no significant increase in cardiac output with either concentration of Arg-Phe (3 ± 2 and 0 ± 3 ml min^{-1}, respectively), despite the concomitant tachycardia (38 ± 7 and 40 ± 10 beats min^{-1}, respectively, $P<0.05$). Indeed, there was a significant decrease in the maximum rate of rise of aortic flow with both concentrations of Arg-Phe (-227 ± 81 and -286 ± 84 l min^{-2}, respectively). It is likely, therefore, that the significant decreases in total peripheral conductance (-226 ± 44 and -314 ± 27 μl min^{-1} mmHg^{-1}, respectively, $P<0.05$) accounted for the increases in mean arterial pressure. In the presence of ICI 118551 and phentolamine or ICI 118551, phentolamine and atenolol, there were significant reductions in the pressor responses to Arg-Phe (15 ± 5 and 24 ± 5 mmHg, respectively; $P<0.01$, compared with control values, analysis of variance). Relative to control responses, the decreases in total peripheral conductance evoked by Arg-Phe were significantly attenuated in a similar manner to the pressor responses, while the cardiac output responses did not differ significantly across the treatment groups ($P>0.05$, analysis of variance).

In other experiments, the pressor effects of Arg-Phe were abolished in pithed rats (Thiemermann *et al.*, 1991) and in ganglion-blocked conscious rats (unpublished observations). One interpretation of the present findings is that the residual pressor and conductance effects of Arg-Phe following adrenoceptor blockade are due to release of neuropeptide Y from sympathetic nerves.

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123P INVOLVEMENT OF β_2-ADRENOCEPTORS IN THE REGIONAL HAEMODYNAMIC RESPONSES TO BRADYKININ IN CONSCIOUS RATS

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In vivo, exogenous bradykinin has complex haemodynamic effects (Thomas *et al.*, 1988; Gardiner *et al.*, 1990a). In the present work we assessed the possibility that release of calcitonin gene related peptide (CGRP) (Gepetti *et al.*, 1988) or activation of β_2-adrenoceptors, consequent upon stimulation of adrenal medullary adrenaline release (Feldberg & Lewis, 1964) contributed to the regional vasodilator effects of exogenous bradykinin.

Male, Long Evans rats (350-450 g) were anaesthetized (sodium methohexitone, 60 mg kg^{-1} i.p. supplemented as required) and had pulsed Doppler probes implanted to monitor changes in renal, mesenteric and hindquarters blood flow (Gardiner *et al.*, 1990a). At least 7 days later, animals were re-anaesthetized (sodium methohexitone, 40 mg kg^{-1} i.p.) for placement of intravascular catheters, and experiments were begun following 1 day recovery. Animals (n=8) received 6 bolus injections of bradykinin (3 nmol kg^{-1}) with each injection separated by at least 20 min. Between the third and fourth injections of bradykinin, human α-CGRP [8-37] was infused (1.5 μmol kg^{-1} h^{-1}) to inhibit the putative actions of endogenous CGRP (Gardiner *et al.*, 1990b). Between the fifth and sixth injections of bradykinin, ICI 118,551 was infused (0.7 μmol kg^{-1} bolus, 0.4 μmol kg^{-1} h^{-1} infusion) to determine the involvement of β_2-adrenoceptors in the responses to bradykinin. An additional 4 animals received saline vehicle between the fifth and sixth injections of bradykinin.

The first injection of bradykinin caused tachycardia (93 ± 4 beats min^{-1}, $P<0.05$, Wilcoxon's test), and rises in mean arterial blood pressure (13 ± 4 mmHg) and renal (10 ± 3%), mesenteric (57 ± 8%) and hindquarters (68 ± 8%) blood flow and vascular conductance (renal, 10 ± 4%; mesenteric, 42 ± 10%; hindquarters 69 ± 10%). However, the mesenteric effects peaked at about 30 s, whereas the maximum renal and hindquarters changes occurred about 90 s after injection of bradykinin. The subsequent 4 injections of bradykinin elicited effects similar to the first. Hence, human α-CGRP [8-37] (given between injections 3 and 4) did not influence the response to bradykinin. However, the sixth injection of bradykinin (i.e. in the presence of ICI 118,551) had an enhanced ($P<0.05$ versus response to fifth injection) pressor effect (24 ± 2 mmHg) and this was associated with reductions in renal (-11 ± 3%) and hindquarters (-16 ± 4%) vascular conductance. However, mesenteric vascular conductance increased (60 ± 12%) as before. Animals receiving saline vehicle infusions between the fifth and sixth injections of bradykinin showed reproducible responses.

The results are consistent with bradykinin causing adrenal medullary adrenaline release (Feldberg & Lewis, 1964), and the latter exerting β_2-adrenoceptor-mediated vasodilator effects, particularly in the hindquarters vascular bed.

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124P CARDIOVASCULAR RESPONSES TO DOPEXAMINE IN ANAESTHETISED AND CONSCIOUS DOGS

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Dopexamine increases heart rate and myocardial contractility and reduces blood pressure. The increased cardiac activity may be due to stimulation of β_1- and/or β_2-adrenoceptors (Bass et al, 1987), inhibition of noradrenaline Uptake (Mitchell et al, 1987) and/or baroreceptor reflex activity in response to the depressor actions of dopexamine. The aims of the present experiments were to assess the importance of Uptake blockade, baroreceptor reflex activity and β_2-adrenoceptor stimulation in the cardiovascular response to dopexamine and to observe the responses to dopexamine in conscious, instrumented dogs.

Arterial pressure and heart rate were monitored in mongrel dogs, anaesthetised with pentobarbitone. Micromanometers were inserted into the left ventricle. The index of myocardial contractility was dP/dt ÷ integrated isometric tension (Goodman et al, 1972). Micromanometers were implanted in the conscious dogs (n=2) at least 4 weeks prior to experiments with dopexamine. Heart rate and contractility were assessed as above. Control dose-response curves to dopexamine infusions were obtained and repeated after injection of amitriptyline (2 mg/kg i.v.) (n=5) and after bilateral vagotomy and sympathetic denervation of the heart (n=5). When the cardiovascular variables had returned to baseline, ICI 118551 (0.2 mg/kg bolus, 0.2mg/kg/hr) was administered and the dose-response curve for dopexamine was repeated.

Dopexamine increased cardiac contractility and heart rate and decreased blood pressure in a dose-dependent manner. Although there was a trend to reduced inotropic and chronotropic responses after amitriptyline, the shifts in the dose-response curves were not significant. Cardiac denervation significantly decreased heart rate ($P<0.005$) and contractility ($P<0.001$) but had no significant effect on blood pressure. Only the chronotropic response to dopexamine was significantly reduced after denervation ($P<0.05$). After β_2-adrenoceptor blockade, both the inotropic and chronotropic dose-response curves to dopexamine were significantly reduced ($P<0.05$). In the conscious dogs, dopexamine increased contractility and heart rate. The increases in heart rate were significantly greater than those in the anaesthetised animals.

The results confirm the positive inotropic and chronotropic actions of dopexamine and its vasodepressor activity. In anaesthetised animals, the fall in blood pressure was largely due to β_2-adrenoceptor stimulation. The positive inotropic effect was predominantly due to direct stimulation of cardiac β_2-adrenoceptors. Both a direct, β_2-adrenoceptor-mediated response and baroreceptor reflex activity contributed to the tachycardia, particularly in the conscious animals. This work was supported by Fisons plc, U.K.

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125P EFFECTS OF CAPTOPRIL ON REGIONAL HAEMODYNAMIC RESPONSES TO ANGIOTENSIN I IN CONSCIOUS RATS

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The haemodynamic effects of exogenous angiotensin I (Ang I) could be due to its conversion to angiotensin II (Ang II) in the circulation or within particular vascular beds. In the former case, treatment with an angiotensin-converting enzyme (ACE) inhibitor should reduce all the regional haemodynamic effects of Ang I proportionately, whereas in the latter case it is feasible ACE inhibition might exert differential effects on responses to Ang I. We examined these possibilites by giving i.v. bolus injections of Ang I (0.3 nmol kg^{-1}) before and after treatment with captopril (60.5 μg kg^{-1} i.v. bolus) in conscious, Long Evans rats (n=9) chronically instrumented with intravascular catheters and pulsed Doppler probes to measure changes in coeliac, mesenteric and hindquarters blood flows. Surgery for probe implantation was in animals anaesthetized with sodium methohexitone (60 mg kg^{-1} i.p.) at least 7 days before catheters were implanted under brief anaesthesia (sodium methohexitone, 40 mg kg^{-1} i.p.). Experiments began 1 day after catheter implantation when baseline variables were recorded for 30 min before Ang I was given. Following 60 min captopril was administered and Ang I injections were repeated 8 and 60 min later. In the presence of captopril there were no significant, sustained changes in haemodynamic variables.

Table 1: Cardiovascular changes (peak) following i.v. Ang I in conscious, Long Evans rats before and after captopril. Values are mean ± s. e. mean.

	Mean arterial pressure (mmHg)	**Doppler shift (%)**			**Vascular conductance (%)**		
		Coeliac	Mesenteric	Hindquarters	Coeliac	Mesenteric	Hindquarters
Control	56 ± 2*	-32 ± 7*	-54 ± 3*	-17 ± 5*	-56 ± 4*	-70 ± 2*	-47 ± 4*
Captopril (8 min)	16 ± 3*†	5 ± 4†	-35± 3*†	4 ± 5†	-9 ± 3†	-43 ± 3*†	-10 ± 5†
Captopril (60 min)	50 ± 1*	-20 ± 4*	-55 ± 2*	-8 ± 6	-46 ± 3*	-70 ± 2*	-38 ± 4*

* P<0.05 versus baseline (Wilcoxon's test); † P<0.05 versus control (Friedman's test)

The marked inhibition of the pressor response to Ang I was accompanied by abolition of the coeliac and hindquarters vasoconstrictor responses to the peptide 8 min after captopril, although substantial mesenteric haemodynamic effects remained. By 60 min after captopril the responses to Ang I were back to control levels. These findings indicate the *in vivo* haemodynamic effects of Ang I might involve local conversion to Ang II, particularly in the mesenteric vascular bed.

126P DOES A RELATIONSHIP EXIST BETWEEN THE ANTIHYPERTENSIVE ACTION AND THE ANGIOTENSIN RECEPTOR ANTAGONIST ACTIVITY OF DuP 753 IN CONSCIOUS RATS?

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The non-peptide angiotensin receptor antagonist, DuP 753, reduces blood pressure in renal artery ligated hypertensive (RALH) rats and inhibits the pressor response to a single dose of angiotensin II (Ang II). Thus, Wong et al. (1990) have suggested that the antihypertensive effect of DuP 753 is a consequence of its ability to antagonise the effects of endogenous Ang II. In order to investigate whether there is a temporal relationship between these phenomena, we measured the antihypertensive effect of DuP 753 in RALH rats, and the extent and duration of DuP 753-induced displacements of Ang II dose-pressor response curves in conscious normotensive rats.

Male, albino rats (250-400g) were anaesthetised (Isoflurane) and hypertension induced by ligation of the left renal artery. Carotid artery catheters were implanted in normotensive and in RALH (5 days after initial surgery) rats, for blood pressure measurement and drug administration.The next day, DuP 753 (1 and 3 mg/kg i.a.) was administered and its effects on resting diastolic blood pressure (DBP) in RALH rats, and Ang II-induced pressor responses in normotensive rats, were measured at various times thereafter. DuP 753 reduced blood pressure in RALH rats, and in normotensive rats caused rightward displacements of Ang II dose-response curves, from which Ang II dose-ratios were derived (Table 1).

Table 1 Effects of DuP 753 in RALH rats and normotensive rats.

	DuP 753 (mg/kg i.a.)	Basal DBP (mmHg)	Hours after dosing 1	3	5	7	24	n
Fall in DBP (mmHg) (RALH rats)	1	155±10	6±6	38±10	51±12	56±11	25±15	6
	3	151±2	34±10	58±9	64±8	63±8	34±15	5
Ang II Dose-Ratio (Normotensive rats)	1	119±4	3.2 (2.2-4.6)	5.3 (3.0-9.4)	6.2 (2.6-15.0)	3.9 (1.9-7.9)	1.9 (0.6-6.3)	5
	3	123±7	23.7 (11.8-47.7)	14.2 (5.2-38.7)	8.0 (2.5-25.1)	7.8 (2.6-23.7)	0.7 (0.4-1.2)	5

Values shown are arithmetic means ± s.e.mean in RALH rats and geometric means (95% confidence interval) in normotensive rats.

DuP 753 produced a progressive dose-related reduction in blood pressure to sub-normotensive levels in RALH rats and this effect was still evident 24h after dosing. In contrast, the displacement of Ang II pressor-response curves was maximal 1-5h after dosing, and virtually absent 24h after dosing. Moreover, there was no significant, temporal correlation between the ability of DuP 753 to reduce blood pressure and inhibit Ang II responses ($P > 0.1$; Pages' test). Consequently, it seems that the hypotensive effect of DuP 753 may not simply be related to its immediate ability to inhibit the effects of Ang II in the plasma compartment.

Wong, P.C., Price, W.A., Chiu, A.T., Carini, D.J., Duncia, J.V., Johnson, A.L., et al. (1990) Hypertension **15**, 823-834.

127P CAPTOPRIL ENHANCES GLUCOSE TOLERANCE IN SPONTANEOUSLY HYPERTENSIVE RATS

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There is increasing speculation that insulin-resistance may be a causal factor in cardiovascular disease. This resistance is associated with a compensatory hyperinsulinaemia and impaired glucose tolerance in humans and animals (Reaven 1988). Established antihypertensive therapy can exacerbate insulin resistance, whereas captopril has been reported to improve glucose clearance in hypertensive patients (Pollare *et al* 1989). Our experiments were undertaken to establish whether captopril similarly affects glucose clearance in a hypertensive rat model.

Male Japanese Okamoto rats were fasted overnight and anaesthetised with "Hypnorm" (Midazolam 5mg/Kg, and Fluanizone 10mg/Kg, i.p.). The trachea, a carotid artery, and a femoral vein were cannulated. Blood samples (0.2 ml) were taken at predetermined intervals via the carotid cannula. For glucose tolerance studies an arterial blood sample was taken immediately prior to i.v. administration of $1.4x10^{-3}$ mol/Kg glucose at t=0 min. Further arterial samples were taken at t=2,5,10,15, and 30 min. Captopril ($2.3x10^{-5}$ mol/Kg), Epicaptopril ($1.15x10^{-5}$ mol/Kg), or saline (1 ml/Kg) were then administered at t=55 min, and the glucose tolerance curve (GTC) repeated commencing at t=90 min. Plasma samples were analysed for glucose concentration using the colourimetric glucose oxidase method of Werner *et al* (1970).The raw data were then manipulated as follows.The initial pre-glucose dose value for each GTC was subtracted from each of the subsequent values for each animal. The new values for the first GTC were then subtracted from the corresponding values of the second GTC for each animal and plotted graphically. Each point represents mean +/- SEM (n=8 for controls, and n=9 for captopril-treated animals). (Figure 1). Captopril treatment significantly increased glucose clearance at 10 minutes ($p<0.01$), and also at 15 and 30 minutes ($p<0.05$). Epicaptopril caused no significant difference in glucose handling at any time point when compared with controls.

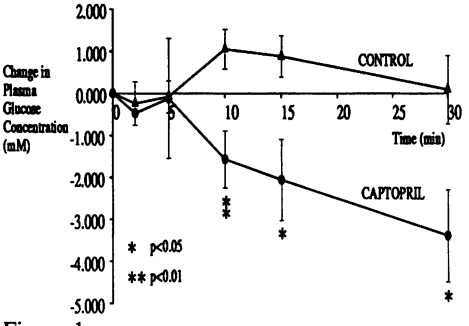

Figure 1.

Captopril has therefore been shown to improve glucose tolerance in hypertensive rats. Its mechanism of action in producing this effect is under investigation.

Pollare, T., Lithell, H., & Berne, C., (1989) *New. Eng. J. Med.***321** (13), 868-873.

Reaven G.M., (1988) *Diabetes* **37**, 1595-1607.

Werner W, H-G Rey, and H Weilinger, 1970. *Z. Analyt Chem* **252**; 224.

DSTC is a SERC-funded scholar.

128P THE HYPOTENSIVE EFFECTS OF A NEW RENIN INHIBITOR IN THE DOG

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The hypotensive efficacy of a new renin inhibitor MDL 73323(N-(3-amino-3-methyl-1-oxobutyl)-o-methyl-l-tyrosyl-n-[1-cyclohexylmethyl3-3-difluoro-4-[(3-methyl-1-oxobutyl)amino]-2-oxobutyl]-l-norvalimide,hydrochloride,hydrate) was tested in three experimental models in conscious male Beagle dogs, treated intravenously (iv) and in one model orally (po).
MDL 73323 showed similar activity, in the nM range, on human,monkey and dog plasma renin activity(PRA).
Three male beagle dogs, treated for six days with a low Na diet and distilled water, received MDL 73323 (30 µg/Kg/min infused iv for 60 minutes). The drug inhibited PRA by 100% during the infusion,but did not affect systemic blood pressure(BP).The PRA returned to basal values two hours after the end of infusion.
Four days after renal constriction five male Beagle dogs (2 Kidney, 1 clip) received MDL 73323 (100 µg/Kg/min infused iv for one hour).During the infusion PRA was inhibited by 97% and systolic (SBP), diastolic (DBP) and mean blood pressure (MBP)were decreased by -13 (7%), -11 (10%), and -12 (9%) mmHg, respectively. After the end of infusion the BP came back to basal value, instead the PRA was still significantly inhibited three hours later.
Three weeks after renal constriction four male Beagle dogs received, Furosemide (120 mg) and a low Na diet, for a period of six days, and thereafter were treated with MDL 73323 (100 µg/Kg/min infused iv for 60 minutes). Again in this model the effect of MDL 73323 on BP was evident during the infusion, but the decrease in BP was greater than in the previous experiment.PRA was inhibited for four hours after the end of infusion, but the peak effect (97%) was attained 60 min after the onset of infusion. SBP, DBP, MBP decreased by -34 (18%), -20 (17%), -24 (17%) mmHg, respectively. Heart rate (HR) increased by 28%. Two months after renal constriction, these four male Beagle dogs again were treated with Furosemide and a low Na diet. MDL 73323 (30 mg/Kg po) was quickly absorbed in these animals and the peak effect on BP, PRA and HR was between 25-30 minutes after the administration of the drug, SBP -22 (12%), DBP -8 (7%) and MBP -17 (10%) mmHg, and HR increased by 49%. PRA was inhibited by 67% at 30 min and remained significantly reduced for over the following 4 h.
In vivo, MDL 73323, is a new and effective antihypertensive drug in the dog.

129P GLUTATHIONE STATUS IN SENSITISED GUINEA-PIG LUNGS

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Glutathione is required for the synthesis of the cysteinyl leukotrienes, which are major candidates for the mediation of bronchial asthma. We have investigated lung glutathione status in an animal model for asthma, and shown a decrease in the reduced glutathione (GSH) content of sensitised guinea pig lungs probably due to a defect in synthesis.

Male Dunkin-Hartley guinea pigs (250-300g) were injected intraperitoneally with 10µg ovalbumin in 20% (w/v) $AlOH_3$ as adjuvant and used for experiments after 14 days. The activity of γ-glutamyl transferase (GT) was measured in lung homogenate, while those of γ-glutamylcysteine synthetase (γGCS) and glutathione-S-transferase (GST) were measured in cytosol. GSH was determined in lung homogenate prepared in 0.2M perchloric acid and protein mixed disulphides (PMD) measured after treatment of homogenates with 5mM dithiothreitol and 8M guanidine.

The GSH content of sensitised lungs was significantly lower than that of controls (1.63 ± 0.02 (n=6) compared with 2.31 ± 0.07 (n=6) µmol GSH/g lung, $p < 0.05$). This decrease in the GSH content of sensitised lungs could be due to (i) oxidation of GSH to the dimer, GSSG, (ii) increased catabolism and/or (iii) decreased synthesis. Oxidation of GSH in cells is accompanied by an increase in intracellular PMD content. There was no evidence for increased formation of PMD in sensitised lung tissue (11.42 ± 0.15 (n=6) µmol GSH equivalents/g lung in control lungs compared with 11.72 ± 0.37 (n=6) in sensitised lungs).

Table 1 Enzyme activities in guinea pig lungs

Enzyme	Control	Sensitised
γGT (nmol/min/mg protein)	11.99 ± 1.34 (6)	8.53 ± 0.73 (8)*
γGCS(pmol/min/mg protein)	54.9 ± 6.7 (6)	21.8 ± 3.9 (8)*
GST (µmol/min/mg protein)	18.25 ± 1.32 (6)	18.63 ± 1.33 (6)

Values are mean ± SEM, with number of animals in parentheses. *$P < 0.05$, by non-paired Student's t test.

Table 1 shows that γGT activity was decreased in sensitised lungs. This is the first enzyme in the catabolic pathway for GSH, and it is also involved in the transport of amino acids (as γ-glutamylamino acids) into cells. Decreased γGT activity may therefore result in a lower intracellular concentration of L-cysteine, the rate limiting precursor for GSH synthesis. Furthermore, γGCS activity was also significantly decreased suggesting that there may be a defect in the synthetic pathway for GSH in the sensitised tissue. The activity of GST measured in the presence of 1mm GSH using the general substrate, 1-chloro-2,4-dinitrobenzene, was not altered by sensitisation. However, decreased availability of GSH in the sensitised lungs *in vivo* may cause defective conjugation.

130P EFFECT OF CROMAKALIM ON VAGALLY-MEDIATED AIRWAY CONSTRICTION IN THE GUINEA-PIG *IN VIVO*

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Potassium activating drugs such as cromakalim may be useful in the treatment of bronchial asthma. Cromakalim attenuates vagally-mediated constriction in isolated guinea-pig trachea (McCaig and De Jonckheere, 1989) and a prejunctional site of inhibition of cholinergic neurotransmission by cromakalim has been proposed (Ichinose and Barnes, 1990). This work aims to demonstrate attenuation of vagal responses by cromakalim in vivo in anaesthetised, ventilated guinea-pigs.

Guinea-pigs (male, 200-300g) were anaesthetised with sodium pentobarbitone (60mgkg^{-1}, i.p.). A carotid artery was cannulated to record blood pressure. Heart rate was derived from the blood pressure trace using a differential amplifier. The trachea was cannulated and air-overflow pressure (AP) measured from a pressure transducer attached to the side-arm in this cannula. Animals were ventilated throughout (stroke volume 8mlkg^{-1} , rate 50 breaths min^{-1}). The right or left vagus nerve was sectioned high in the neck and the distal end placed on a bipolar electrode. Stimuli (25V, 0.5ms duration) were applied for 10s every 2min at frequencies of 2-30Hz. Cromakalim 10, 100 or 1000µgkg^{-1} was administered through a cannula in the right jugular vein 5 min before stimulation of the vagus nerve. Resting AP was 12±0.8 mmHg (mean± s.e.mean, n=6) and vagal stimulation elicited an increase in AP, reflecting airway narrowing, which increased with frequency of stimulation to a maximum at 30Hz (+17±4 mmHg, n=5). In addition heart rate was reduced slightly at frequencies of vagal stimulation up to 10Hz and substantially at 20-30Hz (e.g. 20Hz, -128±19 beatsmin^{-1},n=6). There was also a transient decrease in blood pressure (e.g. at 30Hz, -12±2 mmHg, n=6). Responses were reproducible over time. Cromakalim attenuated vagally-mediated increases in AP in a concentration-dependent manner (e.g. at 10Hz, -17±10%, -35±18% and -65±18% (all n=5) at 10, 100 and 1000µgkg^{-1}, respectively). Responses at lower frequencies of stimulation were affected more profoundly (e.g. -73±13% at 2Hz c.f. -23±11% at 20Hz, 10µgkg^{-1} cromakalim). Both vagally-mediated bradycardia and the decrease in blood pressure were unaffected by cromakalim.

These results indicate that cromakalim effectively attenuates vagally-mediated airway constriction in guinea-pigs in vivo. Cromakalim, therefore, in addition to its direct bronchodilator effect, has the capacity to counteract reflex bronchoconstriction. If these results can be confirmed in humans, cromakalim may be particularly beneficial in patients who previously reqiure both anticholinergic and β_2- adrenoceptor agonist therapy to control their asthma.

McCaig, D.J. & De Jonckheere, B. (1989). Br. J. Pharmacol. **98**, 662-668.
Ichinose, M. & Barnes, P.J. (1990). J. Pharmac. Exp. Ther. **252**, 1207-1212.

131P ROLE OF K^+ CHANNELS IN THE MODULATION OF CHOLINERGIC NEURAL RESPONSES IN AIRWAY SMOOTH MUSCLE

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In airway smooth muscle, neural constrictor responses are evoked by cholinergic and excitatory non-adrenergic non-cholinergic (e-NANC) nerves. These nerves are known to be modulated by various agonists acting on prejunctional receptors, raising the possibility of a common inhibitory mechanism. We have reported that the activation of charybdotoxin (ChTX)-sensitive K^+ channels (K_{ChTX}) may be a common pathway of inhibitory nerve modulation of e-NANC responses in guinea pigs (Stretton, *et al.*, 1991). In the present study, we examined whether the activation of K_{ChTX} are also involved in the inhibitory modulation of cholinergic nerves not only in guinea pig but also in human airways.

Guinea pig tracheal strips or human bronchial strips were suspended in organ baths containing Krebs solution (37°C, pH 7.4) between platinum electrodes for electrical field stimulation (EFS, 40v, 0.5ms, 0.5Hz (guinea pig) or 1Hz (human tissues), for 15s every 4 min). When constant responses to EFS were obtained; 1)effects of ChTX (large conductance Ca^{++}-activated K^+-channel (K_{Ca}) blocker, 10nM) on the inhibition of cholinergic contraction by μ-opioid agonist, D-Ala^2,$NMePhe^4$,Gly-ol^5- enkephalin (DAGO, 0.1μM), α_2-agonist clonidine (10nM), neuropeptide Y (NPY, 0.1μM) and ATP-sensitive K^+-channel (K_{ATP}) opener lemakalim (0.3μM), 2)effects of other K^+ channel blockers, glibenclamide (K_{ATP} blocker, 1μM) and apamin (low-conductance K_{Ca} blocker, 0.1μM), on the inhibitory cholinergic nerve modulation by the same agonists, were examined in guinea pigs. In human tissues, effects of ChTX (10nM) on μ-opioid (DAGO, 0.3μM)-induced inhibition of cholinergic nerves were examined.

In guinea pigs, ChTX (10nM) reversed the prejunctional inhibition of cholinergic contraction by DAGO (67.3±13.1%), clonidine (71.9±22.4%), NPY (84.2±16.2%) and lemakalim (20.9±7%) (n=5, respectively), while apamin (0.1μM) had no effects (n=3). Glibenclamide (1μM) completely reversed lemakalim but not any other agonist-induced inhibition of cholinergic contraction (n=3). ChTX (10nM) itself potentiated cholinergic contraction slightly (24.6±9.4%, n=5), however, the pretreatment of ChTX also significantly reduced the inhibitory modulation of cholinergic nerves by DAGO, clonidine and NPY, indicating the involvement of K_{ChTX} in the inhibitory modulation by these three agonists. In human tissues, ChTX also reversed DAGO-induced inhibition of cholinergic contraction (90.5±5.8%, n=3). These results suggest that the activation of K_{ChTX} are involved in a common pathway of inhibitory modulation of cholinergic nerves in guinea pig and human airways.

Stretton, D., Miura, M., Belvisi M.G. & Barnes P.J. (1991) Am Rev Respir Dis. 142, A755.

132P MODULATION OF EXCITATORY NON-ADRENERGIC, NON-CHOLINERGIC CONSTRICTOR RESPONSES IN GUINEA-PIG BRONCHI BY BRADYKININ

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Bradykinin (BK) is an important inflammatory mediator in the airways, producing bronchoconstriction, the activation of vagal reflexes and an increase in vascular permeability. Airway sensory nerves mediate the excitatory non-adrenergic non-cholinergic (e-NANC) bronchoconstrictor responses and may play a major role in neurogenic airway inflammation. Although BK is known to stimulate sensory nerve endings to evoke vagal reflexes, it is still unclear whether BK play a role in the prejunctional modulation of e-NANC responses. Therefore, in the present study, we examined this hypothesis in guinea pig airways *in vitro*. Furthermore, we also examined whether a specific BK_2 receptor antagonist, HOE140, can inhibit responses evoked by BK in order to elucidate the type of BK receptor involved in the modulation of neural excitatory responses.

Guinea pig bronchial rings were suspended in organ baths containing Krebs solution (37°C, pH 7.4) between platinum electrodes for electrical field stimulation (EFS, 40V, 0.5ms, 8Hz, for 15s). Stimuli were always delivered after the increased tone, produced by BK, had returned to its baseline value. To prevent the breakdown of applied BK by endogenous enzymes, phosphoramidon (1μM) and captopril (10μM) were added, and all e-NANC responses were measured in the presence of atropine (1μM) and indomethacin (1μM). When two consistent control responses were obtained, BK (0.1, 1, 10nM or 0.1μM) was added and a further three stimuli were delivered. In another set of experiments, the effect of BK (0.1μM) on e-NANC bronchoconstrictor response was examined in the presence or absence of HOE140 (0.1μM)[Hock *et al*,1991]. The effect of BK (0.1μM) on exogenous application of substance P (SP, 1nM-10μM) was also examined in guinea pig bronchi in the presence of phosphoramidon (1μM) and captopril (10μM).

BK potentiated e-NANC responses in a dose-dependent manner; potentiation by BK (0.1, 1, 10nM and 0.1μM) was 2.4±2.0%, 10.2±4.6%, 11.9±6.5% and 24.3±7.5%, respectively (n=5). However, BK (0.1μM) had no significant effect on exogenous application of SP; EC_{50} of SP dose-response curves in the presence and absence of BK (0.1μM) was 28.0±8.0nM and 23.3±2.7nM, respectively (n=5). HOE140 (0.1μM) itself had no effect on e-NANC response (n=5), however, administration of HOE (0.1μM) abolished the potentiation of e-NANC response by BK (0.1μM, n=5). These results suggest that BK potentiates e-NANC bronchoconstrictor responses prejunctionally and that this potentiation is mediated by BK_2 receptor.

HOCK,F.J., WIRTH,K, ALBUS,V., LINZ,W., GERHARDS,H., WIENER,G., HENKE,S., BREIPOHL,G., KONIG,W, KNOLLE,J & SCHOLKENZ,B. (1991). *Br.J.Pharmacol*, 102. 769.

133P LIPOXIN A_4 EVOKES AIRWAY MOTOR RESPONSES BY ACTIVATING SENSORY FIBRES: FUNCTIONAL AND NEUROCHEMICAL EVIDENCE

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Lipoxin A_4 (LXA_4) is a lipoxygenase metabolite generated by various inflammatory cells and detected in BAL fluid of patients with lung diseases. It has been proposed that lipoxygenases metabolites contract guinea pig airway also by stimulating fcapsaicin sensitive nerves (Manzini et al., 1989; Manzini & Meini, 1991). The objective of this study was to establish whether LXA_4 may act via release of neuropeptides from sensory nerve endings.

In isolated guinea pig epithelium-denuded bronchial rings, in the presence of indomethacin (5 μM), LXA_4 consistently elicited, in a concentration-dependent manner (1-6 μM), a long lasting contraction. All responses were evaluated in two main bronchi obtained from the same animal, set up in parallel: one for the control cumulative concentration-response curve (CRC) and the other for CRC after treatment. Values were expressed as % of KCl (60 mM)-induced maximal contraction at the higher concentration used. In the presence of neutral endopeptidases inhibitor thiorphan (10 μM for 10 min) a potentiation of bronchoconstriction was observed (from 23±5 to 44±13 %, n=7). In other sets of experiments the pharmacological ablation of sensory afferents by means of capsaicin desensitization (10 μM for 15 min) markedly inhibited the LXA_4 contractile effect from 98±18 to 34±11% (n=6). In order to further characterize the LXA_4 interaction site on capsaicin sensory fibres we studied its effects on bronchial preparation incubated with ruthenium red (10 μM for 20 min), a blocker of receptor-operated membrane cationic channel coupled to the capsaicin receptor: a partial diminuition of the response was observed (from 69±8 to 45±6 %, n=8) while blockade of voltage-dependent Ca^{2+} channels by omega-conotoxin (0.1 μM for 45 min) did not (n=6). Furthermore LXA_4 (6 μM) superfusion of guinea pig isolated bronchi produced an increase in the outflow of CGRP-LI (total evoked release in 30 min being 2028±200 fmol/g wet weight, n=4) which was never observed in tissue previously challenged with capsaicin (10 μM for 30 min).

These data suggest that LXA_4 may behave as a possible endogenous activator of capsaicin-sensitive nerves, becoming a relevant junction-point between lipid mediators and neuropeptides.

Manzini,S., Ballati, L., Geppetti, P., Rubini, I., Meini S, S. & Perretti, F. (1989) Br. J. Pharmac. 98,1077.
Manzini, S. & Meini, S. (1991) Br. J. Pharmac. 103, 1027-1032.

134P DIFFERENT EFFECTS OF NEUROPEPTIDE Y ON NORADRENALINE AND NERVE STIMULATION RESPONSES IN THE RAT ANOCOCCYGEUS MUSCLE

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Neuropeptide Y (NPY) is co-localized and co-released with noradrenaline (NA) from adrenergic nerves. Mechanisms by which NPY exert its effects seem to vary between tissues (Potter, 1988). The present study examined the effects of NPY on adrenergic and non-adrenergic,non-colinergic (NANC) neurotransmission in the rat anococcygeus muscle (AM).

Paired AM were set up as described by Gillespie (1972) for recording of isometric tension. Motor responses to electrical field stimulation (0.25-5 Hz, supramax V, 0.1 msec pulse width, for 10 sec) or to agonists were recorded under a resting tension of 0.5 g wt. NANC relaxations induced by nerve stimulation (0.25-10 Hz, supramax V, 1 msec pulse width, for 10 sec) were also obtained. Tone was raised with Guanethidine (30 μM).

NPY (0.1-0.3 μM) produced a very slight contraction (6.1, 11,20 % relative to maximal contraction of NA) in some but not all preparations. Contractile responses to field stimulation were significantly enhanced ($p<0.05$ and $p<0.01$) by NPY (0.1-0.3 μM) at all stimulation frequencies studied but only the low frequencies (0.25-1 Hz) were potentiated ($p<0.05$ and $p<0.01$) by 0.03 μM NPY.The potentiation of field stimulated responses by NPY was not modified by the presence of DMI (0.1 μM). NPY (0.03 μM) failed to modify the contraction induced by NA in presence of DMI (0.1 μM) and normetanephrine (1 μM). Higher concentrations of the peptide (0.1-0.3 μM) only potentiated ($p<0.05$ and $p<0.01$) the contractions induced by low concentrations (10-30 nM) of NA. In addition, NPY (0.1-0.3 μM) did not modify tyramine-induced contraction of AM. NANC relaxations induced by nerve stimulation at 0.25 to 1 Hz but not those of 2.5 and 5 Hz were significantly inhibited ($p<0.05$ and $p<0.01$) by NPY (0.03-0. 1 μM).

The results obtained suggest that NPY slightly potentiates the postjunctional NA-induced contractile responses in the AM. The higher potentiation observed on nerve stimulation contractile responses could be explained by the NPY-induced decrease of NANC inhibitory response induced by nerve stimulation. However a prejuctional effect of NPY on AM cannot be excluded. More studies should be pursued in order to clarify this effect.

Gillespie, J.S. (1972) Br.J.Pharmac. 45, 404-416.
Potter, E.K. (1988) Pharmac.Ther. 37, 251-273.

135P THE EFFECTS OF ETHANOL ON RESPONSES OF THE RAT ISOLATED VAS DEFERENS TO FIELD STIMULATION

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Ethanol is generally regarded as a non-specific drug, which distributes in and disturbs the functioning of neuronal membranes. Recent evidence suggests that ethanol may act more specifically on membrane bound proteins, particularly those associated with receptor-gated ion channels (Gonzales & Hoffman, 1991). This study compared the effects of ethanol with those of clonidine on responses of the rat isolated vas deferens to electrical field sltimulation. Vasa from Wistar rats (200-250 g) were inserted in silver ring electrodes and suspended in organ baths containing Krebs buffer, gassed with 95% O_2/5% CO_2 at 37°C. The resting tension was set at 2 g. Vasa were stimulated electrically (1-50 Hz, 0.5 ms pulses, supramaximal voltage). Responses to field stimulation and drugs were measured isometrically. Both ethanol (20-100 mM) and clonidine (0.01-0.1 μM) inhibited field stimulation-enhanced contractions in a concentration-dependent manner. This inhibition was most marked with short trains (1-2 s) of low frequency stimulation (2-5 Hz). An important difference between ethanol and clonidine was in their ability to cause a maintained inhibition. Ethanol-induced inhibitions developed quickly and then waned so that the maintained inhibition was less than the initial inhibition but inhibitions to clonidine were well-maintained. The partial recovery from the inhibitory effect of ethanol may reflect diminished release of transmitter and reduced auto-inhibition. Ethanol (100 mM) did not affect responses of the vas to noradrenaline (3-100 μM) or to adenosine triphosphate (10 μM-mM). Clonidine-induced inhibitions were antagonised by yohimbine (μM) but ethanol-induced inhibitions persisted in the presence of yohimbine (μM). The combined inhibitory effect (CIE) of ethanol (100 mM) and clonidine (0.01 μM) administered together was greater than the sum of the individual effects (SIE) on responses to stimulation at 20 Hz (CIE: 38 ± 5%, SIE: 17 ± 2%, mean ± s.e. mean, n=5, 0.05>P>0.01) and 5 Hz (CIE: 68 ± 5%, SIE: 52 ± 3%, n=5, 0.05>P>0.01) but not at 2 Hz. These results suggest that ethanol can inhibit field stimulation-induced release of transmitter in the rat vas and that this action may be synergistic with that of clonidine in certain circumstances.

Gonzales, R.A. & Hoffman, P.L. (1991) Trends Pharmacol. Sc. 12, 1-3.

136P FUNCTIONAL INNERVATION OF THE GUINEA-PIG RENAL PELVIS: ROLE OF SENSORY NEUROPEPTIDES

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We have investigated whether the presence of a functional innervation can be demonstrated in the guinea-pig renal pelvis. The whole renal pelvis was prepared for isotonic recording along the circular axis in isolated organ bath with Krebs solution containing indomethacin (3μM). Circular strips of guinea-pig isolated renal pelvis showed spontaneous phasic contractile activity characterized by a mean frequency of 5.4±0.2 contractions/min. Electrical field stimulation (1-20Hz) evoked a frequency-dependent potentiating effect on amplitude of spontaneous activity, which was unaffected by atropine and guanethidine (3μM each), but abolished by tetrodotoxin (1μM) or by *in vitro* capsaicin desensitization (10μM). Also tachykinins produced a concentration-dependent potentiation, neurokinin A being about 100 times more potent than substance P. The respective EC_{50} and 95% c.l. were 43nM (29-63) and 4μM (2-8.6). On the other hand, rat α-calcitonin gene-related peptide (CGRP) induced a concentration-dependent (1-100nM) inhibition (maximum -60%) of the spontaneous contractile activity of the renal pelvis without decreasing its basal frequency. The neurokinin A(4-10) analog MEN 10,376 (Maggi *et al.*, 1991) (3-10 μM) produced a rightward displacement of the concentration-response curve to neurokinin A without depression of maximal response and suppressed the potentiating effect induced by electrical field stimulation without affecting the response to KCl (20mM). In the presence of MEN 10,376, an inhibitory response was produced by electrical stimulation which was antagonized by the C-terminal fragment (8-37) of human calcitonin gene-related peptide (Maggi *et al.*, 1991). hCGRP(8-37), (0.1μM) antagonized the inhibitory response of exogenously administered CGRP without affecting inhibition induced by isoprenaline (10μM). Capsaicin (10μM) produced a large potentiating effect which approached 80-100% of the maximal response, this functional effect was concomitant whith a marked increase in the outflow of substance P, neurokinin A and CGRP-LI from the superfused guinea-pig renal pelvis.
The present findings indicate the existence of a functional innervation of the circular muscle of the guinea-pig renal pelvis which is entirely dependent upon transmitter release from peripheral endings of capsaicin-sensitive primary afferents.In fact, we found no evidence for cholinergic or adrenergic modulation of pyeloureteral motility. On the other hand, tachykinins and CGRP act as physiological antagonists and, under our experimental conditions, the excitatory action of tachykinins prevail over the inhibitory action of CGRP. Local modulation of renal pelvis motility by sensory nerves could facilitate removal of irritants present in urine, protecting the kidney during obstruction and ureteral antiperistalsis.

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137P EFFECT OF LY 83583 ON THE REPONSE TO NANC NERVE STIMULATION IN RAT ANOCOCCYGEUS AND BOVINE RETRACTOR PENIS MUSCLES

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The relaxation caused by stimulation of the non-adrenergic non-cholinergic (NANC) nerves in rat anococcygeus (RAC) and bovine retractor penis (BRP) muscles results from activation of guanylyl cyclase (Bowman & Drummond, 1984). Pyrogallol and hydroquinone, both generate superoxide anion and inhibit the NANC response of RAC but not BRP muscles (Gillespie & Sheng, 1990). Whether the NANC response of the BRP is mediated by nitric oxide (NO) or another compound which is not sensitive to superoxide anion is not clear. The effects of LY 83583, a potent superoxide anion generator (Furchgott & Jothianandan, 1991), on the response to NANC nerve stimulation in RAC and BRP muscles have therefore been examined.

BRP and RAC muscles were isolated as previously described (Gillespie & Sheng, 1990). RAC and BRP strips were suspended between pairs of Ag-AgCl ring electrodes under 1 g resting tension in 10 ml organ baths, containing Krebs saline at 36°C and gassed with 95% O_2 + 5% CO_2. The adrenergic nerves were blocked and tone raised by addition of guanethidine (30 μM). Field stimulation was applied from a Grass S44 stimulator at supramaximal voltage, 1 ms pulse width, with trains of stimuli lasting 10 s.

Field stimulation between 0.2 and 10 Hz induced a frequency-dependent NANC relaxation in RAC and BRP. LY 83583 (1 μM) inhibited the response to stimulation in both tissues. LY 83583 was more effective in inhibiting the response to NANC nerve stimulation in RAC (55%, 5 Hz, n=6) than BRP (23%, 5 Hz, n=4). Increasing the concentration of LY 83583 to 3 μM further inhibited the NANC response in BRP (51%, 5 Hz, n=4). Superoxide dismutase (100 U/ml) reversed the inhibition produced with LY 83583 (1 μM) in both tissues.

These results, together with our previous data, indicate that the inhibitory response to NANC nerve stimulation in both the RAC and BRP are related to NO formation (Gillespie et al., 1989). The lower sensitivity of the BRP muscle to agents which generate superoxide anions may indicate a higher level of endogenous superoxide dismutase in this tissue.

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138P INCOMPLETE INHIBITION BY ATROPINE OF THE NEURONALLY-MEDIATED CONTRACTILE ACTION OF CICAPROST ON THE GUINEA-PIG ILEUM

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We have shown (Lawrence & Jones, 1988) that the contractile action of the prostacyclin analogue cicaprost on the guinea-pig ileum is abolished by both TTX and morphine: in contrast, contractions produced by iloprost and carbacyclin are only partially inhibited since these prostacyclin analogues have a direct action (blocked by the EP_1-receptor antagonists SC 25191 and AH 6809).

Further studies of cicaprost action (Table 1) were carried out on terminal ileum suspended in Tyrodes solution at 37°C and aerated with 95% CO_2/5% O_2 (isometric recording). Unlike TTX and morphine, atropine at 200 nM failed to abolish contractions (60 - 70% of the ACh maximum) induced by cicaprost. This concentration of atropine abolished matching responses to ACh and produced 95 - 98% inhibition of the twitch response to electical field stimulation (single 1 ms pulse of 40 V every 30 s). SP 150, D-Arg^1,D-3,4-diCl-Phe^5,Asn^6,D-$Trp^{7,9}$,Nle^{11})SP_{1-11} (a relative of spantide from Ferring AB, Sweden) at 1 μM produced a 37% reduction of the cicaprost response and in combination with 200 nM atropine abolished the response. SP 150 markedly inhibited substance P contractions but had no effect on ACh responses; pA_2 on guinea-pig ileum is 7.4 (Ljungqvist *et al.*, 1989) Similar results were obtained for the effects of atropine and/or SP 150 on the neuronally-mediated component of PGE_2 contractions.

Table 1 Effects of TTX, morphine, atropine and SP 150 on prostanoid, ACh and substance P contractions of guinea-pig ileum

	% of control response remaining (mean ± s.e.mean, n = 4 -6)			
Treatment	Cicaprost (13 nM)	PGE_2 (14 nM)	ACh (37 nM)	Substance P (3 nM)
TTX (1 μM)	0	63 ± 3**	-	-
Morphine (1 μM)	0	62 ± 6**	-	-
Atropine (0.2 μM)	34 ±11***	73 ± 1*	0	80 ± 4
SP 150 (1 μM)	63 ± 6**	78 ± 5*	98 ± 2	9 ± 2***
Atropine (0.2 μM) + SP 150 (1 μM)	0	53 ± 5*	-	-

*P< 0.05, **P < 0.01, *** P < 0.001: significant difference from control using Student's t test on % ACh maximum data.

The results indicate that both ACh and possibly substance P mediate the contractile action of cicaprost on the guinea-pig ileum. Further studies on cicaprost action involving other NK-receptor antagonists are in progress.

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139P INHIBITION OF INTESTINAL GABA RESPONSES BY ELECTRICAL FIELD STIMULATION

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We have previously reported that electrical field stimulation (EFS) selectively inhibited $GABA_A$-receptor mediated contractions of intact segments of guinea-pig ileum (Alyami et al, 1990). The inhibition was reversed by washing but was not attenuated by antagonists for a range of putative neurotransmitters or modulators. The extent of the inhibition was dependent on the duration and frequency of EFS. In the present study we further investigate the inhibitory effect.

Longitudinal muscle-myenteric plexus preparations (LMMP) of guinea-pig ileum were suspended in Krebs Henseleit solution (37°C, 95% O_2/5% CO_2 under conditions identical to those previously employed for intact segments. Consistent submaximal isometric contractions were elicited to applications of GABA (0.1mM) administered at intervals of 20 min. Tissues were then subjected to EFS (0.1Hz, 0.2msec, supramaximal voltage) for 2-40 min and the subsequent responses to GABA (0.1mM) were recorded immediately after stimulation. With LMMP, as with intact segments, periods of EFS of 10-40 min caused significant inhibition of GABA contractions ($n \geq 6$), the extent of the inhibition increasing with increasing periods of EFS. After 20 and 40 min periods of EFS, test responses to GABA were 52±3% and 17±3% respectively of control responses. Test responses to GABA were not inhibited by a 2 min period of EFS, or by a 2-40 min EFS-free interval between control and test response or when tissues were washed between the period of EFS and the application of the test dose of GABA. These results obtained using LMMP are not significantly different from those obtained previously using intact segments of ileum.

To establish whether the phenomenon of EFS-provoked inhibition was species-dependent, experiments were performed using intact segments of mouse ileum set up under conditions identical to those employed previously with guinea-pig ileum. Mouse ileum was found to be spontaneously active and to require EFS at a frequency (0.4Hz) which slightly exceeded the rate of spontaneous contractions in order to secure consistent responses. Submaximal contractions induced by GABA (0.1mM) were significantly inhibited by periods of EFS lasting 1-20 min ($n \geq 6$), the extent of inhibition being related to the duration of EFS. For instance, after 5 and 10 min periods of EFS, test responses to GABA were 17±4% and 5±3% respectively of control responses. When intact guinea-pig ileum was stimulated at 0.4Hz, 5 and 10 min periods of EFS reduced GABA responses to 14±5% and 8±2% respectively of control, not significantly different from the inhibitory effect in mouse ileum. In both species EFS for 20 min at 0.4Hz virtually abolished responses to GABA.

The results suggest that the inhibitory effect of EFS upon GABA responses is not dependent on or influenced by the mucosa, submucosa or circular muscle layer of the muscularis externa but involves only those structures present in the LMMP preparation. Furthermore, despite the differing characteristics of mouse and guinea-pig ileum, the phenomenon was demonstrable in both species.

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140P INHIBITORY EFFECTS OF ENANTIOMERIC CANNABINOIDS IN PREPARATIONS OF MOUSE VAS DEFERENS AND OF MYENTERIC PLEXUS-LONGITUDINAL MUSCLE OF GUINEA-PIG SMALL INTESTINE

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Experiments were carried out to compare the potencies of certain enantiomeric cannabinoids as inhibitors of electrically-evoked contractions of vasa deferentia obtained from TO mice and of strips of myenteric plexus-longitudinal muscle dissected from the small intestine of male Dunkin-Hartley guinea-pigs. The cannabinoids studied were (-)-delta-9-tetrahydrocannabinol (THC), (-)-11-hydroxy-delta-8-dimethylheptyl-tetrahydrocannabinol [(-)-DMH], which is markedly more potent than THC as a psychotropic agent, and (+)-DMH which lacks THC-like properties *in vivo* (Little et al., 1989). Vasa deferentia were set up in Mg^{++}-free Krebs solution and the strips of longitudinal muscle in Krebs solution containing hexamethonium bromide (70 μM), mepyramine maleate (0.125 μM) and choline chloride (20 μM). All tissues were mounted in 3 ml siliconized organ baths kept at 37°C and bubbled with 95% O_2 /5% CO_2. Tissues were stimulated at supramaximal current, the vasa deferentia with trains of 3 pulses (train frequency 0.1 Hz; pulse duration 0.5 ms) and the intestinal tissue with single pulses (0.1 Hz; 0.5 ms duration). Isometric contractions were recorded. The cannabinoids were mixed with two parts of Tween 80 by weight and dispersed in saline.

Concentrations of (-)-DMH and THC that decreased twitch heights by 50%, were 1.4 and 60 nM, respectively in the longitudinal muscle preparation (maximum inhibition by both drugs 74 to 80%) and 0.15 and 6.3 nM, respectively in the vas deferens (maximum inhibition by both drugs 85 to 92%). (+)-DMH had no inhibitory effect either in the vas deferens at a concentration of 30 nM or in the longitudinal muscle preparation at a concentration of 100 nM. The vehicle, Tween/saline, also had no inhibitory effect. These results confirm the ability of THC to inhibit the twitch response of the longitudinal muscle of the guinea-pig small intestine and provide further evidence that cannabinoids interact with this tissue through a stereoselective mechanism (see Pertwee, 1988). In addition, they indicate that (-)-DMH and THC are more potent as inhibitors of the twitch response in the mouse vas deferens than in the guinea-pig myenteric plexus-longitudinal muscle preparation and, in line with results from *in vivo* studies (Little et al., 1989), that (-)-DMH is considerably more potent than THC and shows a marked degree of stereoselectivity.

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141P INCREASED RESPONSIVENESS OF GUINEA-PIG GALLBLADDER TO KININS

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Gallbladder strips obtained from Dunkin-Hartley guinea-pigs (400-500g) were suspended in oxygenated Krebs-Henseleit solution at 37°C. Contractions were measured isotonically and expressed as a percentage of responses to 0.1M KCl obtained at the end of each experiment. Responses to successive applications of bradykinin (BK; 0.3 μM; n=6) and the BK_1 selective agonist des-Arg⁹BK (3μM; n=6) increased in a time dependent manner as shown in Table 1. Times given are relative to first establishment of fresh tissue in organ baths.

Table 1.

Time (min)	30	60	90	120	150	180
Response to BK (%)	3±1	26±8	56±7	71±10	78±14	79±6
Response to des-Arg⁹BK (%)	0	0	1±1	3±1	6±4	6±3

This effect was not dependent upon repeated administrations of BK since the response of previously untreated gallbladder strips to BK (0.3 μM; n=11)180 minutes after setting up was 80±13% of KCl induced contractions. Furthermore, the enhanced responsiveness is not a feature of all drugs which produce gallbladder contraction since the response to carbachol (0.1μM) at 30 and 180 minutes was 64±18% and 68±8% respectively. Similar effects of BK have been reported in rat duodenum (Boschov et al.,1984).

Peptidase activity may reduce early responses to BK and gradual depletion of such enzymes would unveil a relatively greater influence of the kinin. To test this hypothesis, responses to BK (0.3 μM) elicited at 60 minutes were studied; a time when either inhibitory or potentiating actions of drugs might be observed. Captopril (10μM; n=8), an inhibitor of angiotensin converting enzyme, significantly increased responses to BK by a factor of 4.1 ($p<0.05$). Inhibitors of enkephalinase (phosphoramidon; 10μM; n=5) or of kininase I (MGTPA; 0.1mM; n=7) did not influence BK stimulated contraction. None of the kininase inhibitors altered contractile responses of gallbladder to PGE_2 (0.1 or 0.3 μM).

The cyclooxygenase inhibitor piroxicam (1μM) reversibly attenuated contractile responses to BK (0.3 μM; n=4) by 87%. In the presence of piroxicam (1μM, which virtually abolished responsiveness to BK) pretreatment with sub-threshold concentrations of PGE_2 (10nM) completely restored the capacity of tissues to respond to BK (0.3μM; n=4). Furthermore, in separate experiments, successive applications of 30nM PGE_2 at 30 minute intervals produced time dependent increases in gallbladder contractions.

In summary, evidence has been provided in support of three mechanisms which may underlie increasing sensitivity of guinea pig gallbladder to kinins. A possible role for recruitment of BK_1 receptors, for depletion of peptidase activity, and for increased levels of (or sensitivity to) endogenous eicosanoids which modulate BK induced contractions have all been observed and each may contribute to the time dependent enhancement of response.

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142P INTERACTION OF GASTRIN/CHOLECYSTOKININ AGONISTS AND ANTAGONISTS ON GUINEA-PIG GALL-BLADDER

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Cholecystokinin contracts gall-bladder smooth muscle and this effect appears to be mediated by CCK_A receptors (Bishop et al., 1990). However, a variety of studies indicate that CCK peptides produce an "extended" concentration-response relationship extending over more than five orders of magnitude of concentration (e.g. Kubota et al., 1985) and this may not be consistent with an interaction at a single receptor type. The aim of the present study was to further investigate the gastrin/CCK receptor(s) that mediate contraction using selective agonists and antagonists.

Guinea-pig gall-bladders were cut along the bile duct axis to provide two preparations. These were mounted in organ baths and bathed in Krebs-Henseleit solution at 37°C gassed with 95% O_2 and 5% CO_2. Contractile responses were measured isometrically. Cumulative concentration-response curves (CRC) to agonists were constructed and were reproducible. Antagonists were allowed to equilibrate for 30 min between CRCs.

Gastrin/CCK peptides stimulated sustained contractions. Caerulein (EC_{50} = 2.2nM) and CCK-8 (EC_{50} = 12.4nM) were the most potent agonists, the remainder (G-17, non-sulphated (ns) G-17, ns CCK-7, ns CCK-8, pentagastrin and CCK-4) were more than 1000 times less potent, consistent with the view that CCK_A receptors mediated this effect. Concentration ratios were obtained for gastrin/CCK antagonists using CCK-8 and caerulein as agonists (Table 1). In contrast to its reported agonist effects in pancreatic acini (Sato et al., 1989) the CCK-8 analogue, JMV-180, exhibited only antagonist activity in gall-bladder (Table 1). The pA_2 estimates for antagonists were similar for both CCK-8 and caerulein, but Schild slope estimates were greater than unity in each case. This may be due to activity additional to gastrin/CCK receptor antagonism, however (with the exception of L-365,260) the lack of effect of high concentrations of antagonists against carbachol (Cch) indicates that these effects are likely to be specific for gastrin/CCK receptors. Whilst both agonist data and antagonism by L-364,718 are consistent with responses being mediated by CCK_A-like receptors, the low apparent affinities of L-364,718 and lorglumide combined with "extended" CRCs for peptides (but not Cch) are consistent with the view that more than one CCK receptor may mediate the contractile responses in gall-bladder. In addition comparisons of binding Kd values in rat pancreas (2.2nM; Sato et al., 1989) and bovine gall-bladder (0.96nm; Molero et al., 1990) suggest that JMV-180 interacts with a different receptor in guinea-pig gall-bladder.

Table 1 Apparent affinity estimates of antagonists against CCK-8 and caerulein-induced contractions in gall-bladder

	CCK-8		Caerulein		Cch
Antagonists	pA_2	slope	pA_2	slope	pK_B
L-364,718	8.61 (8.39-8.92)	1.46 (1.18-1.75)	8.74 (8.57-8.97)	1.44 (1.21-1.67)	<7.0
L-365,260	6.18 (5.84-7.53)	1.29 (0.46-2.14)	6.8*		5.90
Lorglumide	6.10 (5.71-7.13)	2.10 (0.09-3.10)	7.4*		<5.5
JMV-180	6.66 (6.35-7.24)	1.65 (1.11-2.19)	6.19 (6.49-7.79)	1.47 (0.91-2.03)	<5.5

Values shown are pA_2 and Schild slope estimates with 95% confidence interval. * Denotes pK_B values calculated at a single antagonist concentration (n = 3-25).

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143P LOCATION OF PUTATIVE 5-HT_4 RECEPTORS IN RAT OESOPHAGUS

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5-HT_4 receptors mediate relaxation of rat oesophageal tunica muscularis mucosa (T.M.M.) (Baxter et al. 1990). In another 5-HT_4 receptor model, the electrically stimulated guinea pig ileum, the 5-HT_4 receptor is located on post-ganglionic neurones (Craig and Clarke; 1989). In the rat oesophagus, experiments involving tetrodotoxin (TTX) suggest that the 5-HT_4 receptor is located on smooth muscle (Baxter et al. 1991). However, since tetrodotoxin blocks only the process of nerve conduction, it remains a possibility that the 5-HT_4 receptor could release neurotransmitters by directly depolarising nerve terminals. This study was undertaken to clarify the location of the 5-HT_4 receptor in rat oesophagus. Rat oesophageal T.M.M. were set up using a modification of the Baxter technique (Baxter et al. 1990) Cumulative concentration-response curves to 5-HT ($3x10^{-10}$ - $3x10^{-6}$M 5-HT) were constructed. Tissues were then washed with Kreb's containing either 1 µM TTX and 25 mM Mg^{2+}, 1mM Ca^{2+} (Solution 1) or 1 µM TTX and 12 mM K^+ (Solution 2). All bathing solutions were isotonic (318mM). Following 90 min incubation the concentration-response curves to 5-HT were repeated. Results were expressed as mean EC_{50} ± S.E; and maximum % relaxation ± S.E; Values were compared using paired t-test ($P<0.05$ = significance). Incubation with either Solution 1 or Solution 2 had no significant effect on the 5-HT concentration-response curve (see Table 1). The high Mg^{2+}, low Ca^{2+} solution (Solution 1) in combination with TTX blocks both spontaneous and electrically-evoked neuronal transmission whilst the high K^+ solution (Solution 2) will depolarise nerve endings and block neuronal transmission. Because the 5-HT response is unaffected by either Solution 1 or Solution 2, this would suggest that 5-HT_4 receptors in the rat oesophagus are not located on neurons but on smooth muscle.

TABLE 1

	CONTROL	SOLUTION 1	SOLUTION 2	
n	8	4	4	
	Kreb's	25mMol Mg^{2+}, 1mMol Ca^{2+} + 1µMTTX	12mMol K^+ + 1µMTTX	
EC_{50} (nM)	30 ± 8.03	38±6.25	41 ± 9.12	
Max%	80.9±4.01	77.9±9.06	77.7±2.08	**P>0.05 in all cases**

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144P *IN VITRO* STUDY OF THE EFFECT OF SALMON CALCITONIN UPON OPIOID RECEPTORS

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The mechanism by which salmon-calcitonin (S-CT) produces analgesic effects has not yet been evaluated, nevertheless, it has been suggested that the opioid system may be implicated. Previous studies in myenteric plexus-longitudinal muscle strips (MP-LP) from guinea-pig ileum, have shown that, when tissues were incubated with S-CT, the inhibitory effect of morphine was significantly increased. These results suggest that some of the analgesic effects of S-CT could be attributed to an up-regulation of the opioid receptors.

The present study was undertaken to determine which of the opioid receptors could be involved in the effect of S-CT. μ-receptors were tested in the MP-LM strips preparations, DAMGO was used as a μ-selective agonist; δ-receptors were tested in mouse vas deferens, Met-enkephalin (Met-Enk) was used as a δ-agonist and κ-receptors were tested in rabbit vas deferens, bremazocine was used as a κ-agonist drug. S-CT (0.1 U/ml) was added to the organ bath 30 min before opioid drugs were administered. Results obtained after DAMGO or bremazocine addition are summarized in the table.

Table: % inhibition of the contractile response induced by cumulative addition of DAMGO or bremazocine in control tissues and in S-CT pretreated tissues.

DAMGO[μM]	Control	S-CT	Bremazocine [nM]	Control	S-CT
0.01	28.1 ± 9.3	39.1 ± 8.6	1	10.9 ± 2.9	24.6 ± 4.7*
0.02	43.6 ± 9.3	54.5 ± 9.3	2	25.0 ± 2.8	47.8 ± 6.6*
0.04	58.8 ± 8.8	67.7 ± 10.0	4	48.0 ± 6.0	71.6 ± 5.5*
0.08	73.9 ± 6.1	76.5 ± 9.4	8	75.4 ± 4.9	93.2 ± 3.3*

Data are mean values ± s.e. mean (n=8). *$p< 0.01$ *vs* control by ANOVA.

The effect on the inhibition induced by Met-Enk was tested by administration of a single dose of the drug (0.03 μM) and measured 1 and 10 min after Met-Enk administration. 1 min after Met-Enk the inhibition was in control: 37.7±4.4 and in S-CT pretreated: 51±6.3*, 10 min after in control: 11.8±2.2 and in S-CT pretreated: 36.2±5.4* (n=8). Our results suggest that κ- and δ-receptors may be involved in the analgesic effect of S-CT.

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145P EFFECT OF NEWLY DEVELOPED TACHYKININ AGONIST AND ANTAGONISTS ON THE GUINEA-PIG ISOLATED GALLBLADDER

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Tachykinins (TKs) are a family of peptides which share a common C-terminal sequence. In the isolated guinea-pig gallbladder TKs exert a potent contractile effect which, by the use of natural and synthetic TK agonists, has been shown to involve activation of NK_2 and possibly NK_3 receptor types (Maggi et al., 1989). Recently new potent receptor TK antagonists selective for the NK_2 receptor have been synthesized. The introduction of the newly developed NK_2 receptor antagonists has been instrumental for the characterization of NK_2 receptor subtypes, preliminary termed NK_{2A} and NK_{2B}, in several mammalian tissues (Maggi et al.,1991 in press, Van Giersbergen et al,1991). The aim of this work was to further characterize the nature of the tachykinin receptors present in the guinea-pig gallbladder, either by the use of the newly developed antagonists MEN 10,376 (a close analog of MEN 10,207 with reduced partial agonist activity) L 659,877 and R 396, or by the use of MDL 28,564, a pseudopeptide which has been shown to exert diverging actions in tissues endowed with NK_2 receptors (Buck et al., 1990). The experiments were performed on isolated longitudinal strips of guinea-pig gallbladder in the presence of a mixture of peptidase inhibitors (thiorphan, bestatin and captopril 1 μM each) in order to reduce peptide catabolism. All the antagonists tested competitively antagonized the contractile effect of NKA with the following rank order of potency: MEN 10,376 (pA_2= 6.81; slope= -0.78) > L 659,877 (pA_2= 6.35; slope = -0.85) > R 396 (pA_2= 5.26; slope = -0.95). MDL 28,564 acted as a full agonist, its maximal response being 97±8 % of that to NKA. MDL 28,564 was about 30 times less potent than NKA (EC_{50} = 1.86±0.7 μM and 61.8 ±13.3 nM, respectively). This picture was the same of that observed in other isolated preparations endowed with NK_2 receptors, like the endothelium-deprived rabbit pulmonary artery, the circular muscle of the human ileum or the guinea-pig bronchi, which have been claimed to contain the NK_{2A} receptor subtype, while an opposite picture was observed in other preparations like the hamster trachea or hamster urinary bladder which have been claimed to contain the NK_{2B} subtype (Maggi et al.1991 in press).

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146P AN EXAMINATION OF THE SELECTIVITY OF A NEW NON-PEPTIDE TACHYKININ ANTAGONIST

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The substituted quinuclidine CP-96,345 {(2*S*, 3*S*)-*cis*-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo [2.2.2]octan-3-amine} has recently been described as the first non-peptide antagonist for a tachykinin receptor (Snider *et al.*, 1991). In the light of the claimed specificity of this compound for the NK_1 type of tachykinin receptor, we have been interested in testing, using the (2*S*, 3*S*) and (2*R*, 3*R*) racemic mixture, its selectivity in *in vitro* pharmacological assays.

Responses to tachykinins were examined using the selective agonists [Sar^9, $Met(O_2)^{11}$]SP, [β-Ala^8]NKA(4-10) and senktide for the NK_1, NK_2 and NK_3 receptors respectively. NK_1 responses were obtained in the myenteric plexus-longitudinal muscle preparation (MPLM) from guinea-pig ileum, guinea-pig trachea, and rabbit jugular vein, NK_2 responses in guinea-pig trachea, rat colon and rabbit pulmonary artery, and NK_3 responses in guinea-pig MPLM and rat portal vein.

In the guinea-pig trachea and rabbit jugular vein the response to the NK_1 agonist was blocked by the racemic antagonist, apparently in a competitive manner (pK_B 6.9 and 9 respectively) while the response to the NK_2 agonist in the trachea was unaffected. The lack of effect at the NK_2 receptor was confirmed in the rat colon and the rabbit pulmonary artery. The compound was a powerful blocker of the NK_1 response in the MPLM, but also blocked the NK_3 response to senktide; in either case the blockade was not competitive. As in the MPLM the response to senktide in the rat portal vein was blocked, with the effect seen as a marked reduction of the maximum response.

The effect of the antagonist on the response to senktide is not associated with a direct effect at the NK_3 receptor recognition site and is not seen in the MPLM when the effect of senktide is measured as the stimulation of PI hydrolysis (Watling & Guard, personal communication), but is due to some non-specific action since contractile responses of the portal vein to noradrenaline or carbachol, or the responses of the MPLM to histamine or carbachol are also affected, and in a qualitatively similar manner. Interestingly, a similar non-specific action is seen in these preparations with the structurally related compound hydroquinidine.

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147P THE PRESENCE OF NK_3 TACHYKININ RECEPTORS ON RAT UTERUS

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The existence of three tachykinin receptor subtypes was originally proposed on the basis of agonist potency differences in bioassays and in radioligand binding studies and has since been confirmed by the cloning of each receptor. Although the NK_3 receptor was first identified on myenteric plexus neurones of the guinea pig ileum, it is found predominantly in the CNS and has only been described post-synaptically on rat portal vein. The receptor is most easily identified by the activity of the highly selective agonist succinyl-[Asp^6,N-$MePhe^8$]-SP(6-11) (senktide); no antagonist has been described. Radioligand binding studies with [^{3}H]-senktide indicate that NK_3 receptors in the CNS are very similar, if not identical, to those in guinea-pig ileum. The present study describes the presence of a postsynaptic NK_3 receptor on a second peripheral tissue, the rat uterus.

Senktide induced a rapid contraction peaking within 15 - 30 s which was maintained for at least 2 min. The EC_{50} for contraction was 180 ± 77 nM and the maximal response (~2 g) was similar to that seen with oxytocin. Contraction to a sub-maximal concentration of senktide was not altered in the presence of 10 μM L-659877, an NK_2 antagonist, or [D-Pro^4,D-$Trp^{7,9,10}$]SP(4-11) (PTTT), an antagonist at NK_1 and NK_2 receptors. These data strongly suggest the presence of an NK_3 receptor in this tissue. Contractions to senktide were not altered in the presence of tetrodotoxin (300 nM), indomethacin (20 μM) or atropine (1 μM) suggesting that the receptor is located on the smooth muscle cells. Senktide-stimulated a maximal increase in total [^{3}H]inositol phosphates of approx. 30 % above basal in cross-chopped slices of rat uterus during a 30 min incubation with an EC_{50} of 190 ± 99 nM.

The presence of other tachykinin receptors on the rat uterus was investigated. The NK_2 selective agonist [β-Ala^8]NKA(4-10) induced a potent contraction; the response to 100 nM [β-Ala^8]NKA(4-10) was inhibited completely by 10 μM L-659877. The EC_{50} of [β-Ala^8]NKA(4-10) for contraction was 25 ± 5 nM. Substance P methyl ester also induced a potent contraction, EC_{50} 36 ± 6 nM, although its maximum response was 35 ± 5 % of that to senktide or [β-Ala^8]NKA(4-10).

Competition studies with tachykinins and their analogues against [^{125}I]BHSP, [^{125}I]NKA and [^{125}I]BHE gave three distinct rank orders of potency confirming the presence NK_1,NK_2 and NK_3 receptors in the uterus.

In conclusion, this study describes NK_3 tachykinin receptors which appear to be located on smooth muscle cells of rat uterus. Although this tissue also contains NK_1 and NK_2 receptors, its accessibility together with the existence of a selective agonist, senktide, may make it a useful tissue for the evaluation of NK_3 antagonists.

148P ACTIONS OF VARIOUS NEUROMEDIN U FRAGMENTS ON THE SMOOTH MUSCLE OF THE ISOLATED RAT UTERUS

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Neuromedin U (NmU) was originally isolated from porcine spinal cord by Minamino et al. (1985), in two molecular forms NmU-25 and NmU-8 (25 and 8 amino acids, respectively). It was characterised by its ability to contract the rat uterus *in vitro*. Porcine NmU-8 is biologically active and it is known that the amide structure at the C-terminal end is essential for biological activity.

We therefore aimed to identify the shortest NmU fragment that was capable of biological activity; and the contractile activity on the rat uterus of NmU(4-8), NmU(3-8), NmU(2-8) and Phe^1-NmU-8 (custom synthesis; Palliard Chemical Company Ltd, Twinstead, Sudbury, Suffolk, UK) was studied.

Female Wistar rats (150-200 g) were used. The animals were sacrified by asphyxiation. The uterus was removed, mounted in an organ bath and maintained in Krebs solution at 30°C and pH 7.4, aerated with 95% O_2/5% CO_2. The tissues were equilibrated under a resting tension of 0.5 g and the isotonic muscular contractions were recorded as previously described (Domin et al., 1989).

Each fragment was tested in the dose range 10^{-10} M to 10^{-7} M. Porcine NmU-8 (custom synthesis; IAF Bio-Chem, Quebec, Canada), NmU(2-8) and Phe^1-NmU-8 evoked contractions in a concentration dependent manner.

The fragment NmU(2-8)(EC_{50}=6.6±0.2 nM) was 10 times less potent than porcine NmU-8(EC_{50}=0.62±0.03 nM). The effect of Phe^1-NmU-8(EC_{50}=0.55±0.09 nM) showed the same magnitude of response as that obtained with porcine NmU-8. The fragments NmU(3-8) and NmU(4-8) were found to be inactive on the rat uterus.

The results indicate that the C-terminal fragment NmU(2-8) constitutes the minimum length required to activate the smooth muscle of the rat uterus. However, this fragment is not as potent as porcine NmU-8 and it therefore seems that an extension at the N-terminal end is required for full biological activity. This study adds to the knowledge of the bioactive structure of NmU.

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149P NK_1 AND NK_2 NEUROKININ RECEPTORS CHARACTERIZED IN THE RAT ISOLATED URINARY BLADDER PREPARATION

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The neurokinin receptor subtypes mediating contraction of the rat urinary bladder *in vitro* are still unresolved. With the recent availability of novel agonist and antagonist subtypes selective ligands, including those within the NK_2 category (Maggi *et al.*, 1990), re-investigation of this problem has become opportune. We have compared a number of novel agonist and antagonist neurokinin ligands to throw further light on the question of subtypes within this preparation. In two series of experiments summarised in Table 1, potencies of NK_1 or NK_2 selective agonist ligands (Hagan *et al.*, 1991: see, Maggi *et al.*, 1990) were assayed against substance P. These potency estimates were unchanged by the presence of the peptidase inhibitors phosphoramidon and enalaprilat (1 μM) ($P > 0.05$). pD_2 values are mean (± s.e.mean) for 4-6 preparations.

Table 1 AGONIST	pD_2 (± s.e.mean) With Inhibitors	Without Inhibitors
Substance P	6.39 (0.11)	6.65 (0.30)
Neurokinin A	6.79 (0.10)	7.05 (0.17)
Neurokinin B	6.08 (0.20)	6.29 (0.15)
GR64349	6.59 (0.30)	6.50 (0.20)
[β-Ala^8]-NKA$_{(4-10)}$	6.09 (0.23)	6.51 (0.25)
Substance P	6.26 (0.12)	6.03 (0.24)
Substance P methyl ester	6.07 (0.13)	5.70 (0.29)
GR73632	6.28 (0.22)	6.23 (0.18)

In antagonist studies, the NK_2 selective antagonists Ac-Leu,Met,Gln,Trp,Phe,Gly.NH_2 L-659,874 (Williams *et al.*, 1988) and [Tyr^5, D-Trp^{6,8,9}, Arg^{10}]-NKA$_{(4-10)}$ (MEN10207, see Maggi *et al.*, 1990) competitively antagonised responses to the NK_2 selective agonist [β-Ala^8]-NKA$_{(4-10)}$ (pK_B estimates were 6.61 ± 0.08 and 5.75 ± 0.06 respectively, n = 9-11). The NK_2 selective antagonist L-659,874 antagonised responses to the neurokinin receptor non-selective agonist neurokinin A. The resulting Schild regression was alinear suggesting the presence of a mixed receptor population.

These results are discussed in relation to the co-existence of NK_1 and NK_2 subtypes.

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150P COMPARISON OF 5-HT RECEPTORS IN OESOPHAGEAL MUSCULARIS MUCOSAE IN VARIOUS SPECIES, *IN VITRO*

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The internal muscularis mucosae of the oesophagus is sensitive to 5-HT (Bartlet, 1968). Species differences may exist since 5-HT contracts guinea-pig and chicken oesophageal muscularis mucosae (OMM) but relaxes rat OMM (Bartlet, 1968). Baxter et al., (1991) have shown recently that 5-HT-induced relaxation of rat OMM were mediated by putative 5-HT_4 receptors, a subtype distinct from $5\text{-HT}_{1\text{-like}}$, 5-HT_2 and 5-HT_3 receptors. The aim of the present study was to further characterize the 5-HT receptor subtypes in OMM from guinea-pig, rat, cat and ferret.

The OMM from each species were prepared for isolated tissue recording using the method of Baxter et al., (1991). Parallel experiments were performed in which either contractile or relaxant responses (undertaken in tissue precontracted with 10 μM carbachol) to 5-HT were studied. Cumulative concentration-response curves to 5-HT were constructed for rat OMM, whereas non-cumulative curves were constructed in the other species. The propensity of the guinea-pig tissue to desensitize to subsequent additions of 5-HT, necessitated the use of a 20 min dose-cycle, following an agonist exposure period of 1 min. All values quoted are mean, s.e. mean <10%, from 6 animals.

5-HT (0.1 μM-100 μM) elicited a concentration-dependent contractile response in guinea-pig OMM (-log EC_{50} = 5.24), but not in the rat, cat or ferret OMM. In precontracted tissue, relaxant responses to 5-HT (-log EC_{50} = 7.93) were observed only in the rat, but not in the remaining tissues. Tetrodotoxin (1 μM) and atropine (0.1 μM) abolished responses in guinea-pig OMM but were without effect in the rat OMM. Methysergide (1 μM) and ketanserin (1 μM) were without effect in both preparations, whilst ondansetron (10 μM) antagonized responses in the guinea-pig (-log K_B = 7.0) but not in the rat. Partial agonist responses in guinea-pig OMM were seen only with 2-methyl-5-HT (-log EC_{50} = 4.34). In contrast, relaxant responses to 5-HT in rat OMM were seen with 5-methoxytryptamine, (S)zacopride, (R,S)zacopride, (R)zacopride and metoclopramide (-log EC_{50} = 7.70, 7.32, 7.02, 6.38, 5.62, respectively). These responses were competitively antagonized by the putative 5-HT_4 antagonist SDZ 205,557 (2-diethylamino ethyl-(2-methoxy-4-amino-5-chloro)benzoate; Buchheit and Gamse, 1991) with -log K_B values ranging from 7.3-7.8.

In guinea-pig OMM, the data are consistent with a 5-HT_3 receptor, located neuronally, to elicit acetylcholine-induced contractions whereas, in rat OMM, a postjunctional, putative 5-HT_4 receptor appears to mediate relaxations. The lack of effect of 5-HT in cat and ferret OMM argues for a species-dependent role for 5-HT in oesophageal motility.

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151P PUTATIVE 5-HT$_4$ RECEPTOR INVOLVEMENT IN THE RELAXATIONS INDUCED BY 5-HT IN THE RAT ILEUM

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5-Hydroxytryptamine (5-HT) has been shown to mediate contractile responses in the guinea pig ileum via neuronally located 5-HT$_3$ and putative 5-HT$_4$ receptors (Craig and Clarke 1990). In the present study we investigate the actions of 5-HT to mediate relaxation responses in the rat ileum and provide a preliminary characterisation of the receptor involvement.

Isolated ileal segments (2 cm, taken approximately 3 cm from the ileo-ceacal junction) were obtained from female Hooded Lister rats (200-250 g) and mounted in 10 ml organ baths containing oxygenated (95% O_2 / 5% CO_2) Krebs-Henseleit solution (37°C) usually containing methysergide (1 μM) and atropine (0.1 μM) under an initial tension of 1g. The tissues were allowed to equilibrate for 45 min and concentration response curves to 5-HT and other agonists were obtained in a cumulative manner using a 2 min contact time. A comparison of the effects of agonists and antagonists was carried out using paired preparations.

In the absence of methysergide and atropine, 5-HT induced inconsistent changes in muscle tension. In the presence of methysergide and atropine, concentration dependent relaxations were observed with 5-HT (10^{-8}-10^{-6} M, the relaxations decreasing at higher concentrations of 5-HT ($3x10^{-6}$-$3x10^{-5}$)), renzapride, and 5-methoxytryptamine (-log EC_{50}±s.e.mean values were 7.16±0.04, 6.29±0.12 and 4.87±0.09 respectively). 2-Methyl-5-HT caused relaxations only at a concentration greater than 30 μM. The response to 5-HT was not affected by ondansetron (1 μM) or ritanserin (0.1 μM) but response curves to 5-HT exhibited a dextral shift in the presence of ICS 205-930 (figure 1). The pA_2 value for ICS 205-930 was calculated as 6.03.

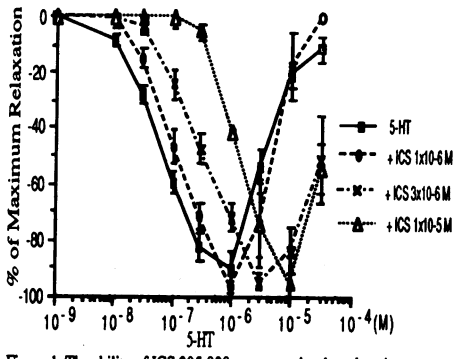

Figure 1. The ability of ICS 205-930 to antagonise the relaxation response induced by 5-HT in the rat ileum.

The failure of methysergide, ritanserin and ondansetron to antagonise the 5-HT induced relaxations indicates that the effect is not mediated via 5-HT$_1$, 5-HT$_2$ or 5-HT$_3$ receptors. The agonist actions of renzapride and 5-methoxytryptamine, and the ability of micromolar concentrations of ICS 205-930 to antagonise the 5-HT induced response, is indicative of the relaxation response being mediated via a 5-HT$_4$-like receptor.

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152P β-ADRENOCEPTORS MEDIATING RELAXATION IN HUMAN COLON *IN VITRO*

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Both α- and β- inhibitory adrenoceptors are present postjunctionally in human colonic circular smooth muscle (McLaughlin *et al.*, 1988). The present experiments were carried out to further characterize the β-adrenoceptors using selective agonists and antagonists.

Circular strips of macroscopically normal segments of human colon (obtained fresh from operation and stored overnight at 4°C) were suspended in Krebs solution at 37°C containing cocaine (3 μM), EDTA (30 μM) and ascorbic acid (30 μM). The Krebs solution also contained phentolamine (1 μM) to block α-adrenoceptors. After an initial equilibration period of approximately 30 minutes, the strips exhibited spontaneous myogenic phasic activity, typically of around 2g amplitude. Cumulative concentration response curves to agonists were carried out and response expressed as % relaxation.

Isoprenaline, noradrenaline and adrenaline inhibited the spontaneous activity of the strips with an order of potency of isoprenaline (1.0) > adrenaline (0.06) > noradrenaline (0.014). Salbutamol (0.01-30 μM) failed to inhibit spontaneous activity in 11 smooth muscle strips from 6 specimens of human colon although isoprenaline produced inhibition in 15 strips from the same specimens (pD_2, 6.34±0.27). In a separate series of experiments, the β$_2$-adrenoceptor antagonist, ICI 118,551 (1 μM), had no effect on responses to isoprenaline while the β$_1$-adrenoceptor antagonist, betaxolol (1 μM), significantly shifted the concentration response curve of isoprenaline to the right with a pK_B of 7.72 ± 0.22 (pD_2 values of isoprenaline, n =6: control, 6.39 ± 0.29; ICI 118,551, 6.32 ± 0.32; betaxolol, 4.78 ± 0.36). The atypical β-adrenoceptor agonist, BRL 37344, had no effect on the spontaneous activity of 10 strips from 3 specimens of colon although in four strips from these same specimens isoprenaline did inhibit the spontaneous activity with a pD_2 of 6.94 ± 0.15.

The lack of effect of salbutamol on spontaneous activity and the lack of antagonism of isoprenaline by ICI 118,551 suggest no involvement of β$_2$-adrenoceptors in the inhibition of spontaneous activity. The lack of effect of BRL 37344 also suggests a lack of involvement of atypical β-adrenoceptors in relaxation, which is in contrast to rat colon (Bianchetti & Manara, 1990; McLaughlin & MacDonald, 1990). The effects of betaxolol on isoprenaline-induced inhibition of spontaneous activity suggest that the β-adrenoceptors in the human colonic circular smooth muscle are of the β$_1$-subtype.

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153P CHOLECYSTOKININ-INDUCED INTESTINAL MOTILITY IN THE ANAESTHETISED DOG: A NOVEL MODEL FOR EVALUATING INHIBITORS OF INTESTINAL MOTILITY

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Cholecystokinin octapeptide sulphated (CCK-8s) stimulates canine antral motility via an atropine sensitive, but hexamethonium resistant pathway; this is consistent with CCK-8s acting indirectly via a post-ganglionic cholinergic neuron (Kuwahara et al,1986). However, in other regions of the canine GI tract the ability of atropine to block CCK-8s is variable (Huizinga et al,1984). This abstract assesses the utility of CCK-8s as a stimulant of both small intestinal (SI) and colonic motility in the anaesthetised dog, as a model to evaluate pharmacologically distinct inhibitors of intestinal motility.

Male beagle dogs (11-14.5 kg) were fasted overnight, premedicated with Dipidolor (4mg kg^{-1} sc Janssen) and anaesthetised with chloralose/urethane (0.08g kg^{-1}-0.8g kg^{-1}). Animals were artificially ventilated and cannulae inserted for measurement of blood pressure, heart rate and compound administration. Cardiac output was determined using a thermodilution method, allowing calculation of total peripheral resistance (TPR). Cardiac contractility was derived from left ventricular pressure, measured using a pig tail catheter. Intestinal motility was measured by Millar micro-tip pressure transducers located either in the jejunum at a point 10cm distal to the ligament of Treitz or in the colon at point 10cm oral to the anal sphincter. Motility was stimulated by an infusion of CCK-8s at 75-100ng kg^{-1} min^{-1} for 9 minutes. Compounds were given by infusion and results are expressed as doses (μg kg^{-1} min^{-1}) that produce a 50% reduction in motility or TPR (ID_{50}) or a 50% increase in heart rate (ED_{150}).

SI motility was inhibited by the α_2 agonist UK-14,304 (IC_{50} 0.04μg kg^{-1} min^{-1}, n=3) and the Na^+ channel blocker tetrodotoxin (25μg kg^{-1}). These results are consistent with CCK-8s acting neuronally to produce an indirect stimulation of motility. Nifedipine, a calcium antagonist, potently inhibited motility in the SI and colon, accompanied by marked cardiovascular effects (Table 1). Atropine, a muscarinic antagonist, potently inhibited motility in the SI and the colon, with a concomitant tachycardia (Table 1).

Table 1	ID_{50} SI Motility	ID_{50} COLON Motility	ID_{50} TPR	ED_{150} Heart Rate	ID_{25} MBP
Nifedipine (n=4)	1.5	1.7	1.0	1.15	10
Atropine (n=4)	0.63	0.78	>10	0.85	>10

We conclude that CCK-8s acts indirectly, via the enteric nevous system, to stimulate motility and this can be inhibited by α_2 receptor activation. The potent inhibition of both SI and colonic motility by atropine and nifedipine shows clearly that CCK-8s acts via the release of acetylcholine and the response is dependent on the entry of extracellular calcium. Therefore, we propose that this is a suitable model for evaluating the activity of various inhibitors of gut motility on the GI tract and the cardiovascular system.

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154P THE ACTIONS OF SOME MUSCARINIC ANTAGONISTS ON DIGESTIVE JEJUNAL MOTILITY IN THE CONSCIOUS DOG

B.McRitchie & Paula Memer introduced by M.G.Dodd, Pfizer Central Research, Sandwich, Kent, CT13 9NJ

The identity and role of muscarinic receptor subtypes involved in the control of gut motility is still unclear. Results from radioligand binding experiments show that intestinal muscarinic receptors are mainly of the M_2-subtype indistinguishable from those in the heart while in vitro tissue bath studies indicate that the muscarinic receptors mediating contraction are of the M_3-subtype (Lazareno & Roberts 1989). There is also evidence for a functional inhibitory role for receptors of the M_1-subtype in the control of gut motility (Fox et al 1985, Schiavone et al 1988). Experiments were conducted to try to further elucidate the functional roles of muscarinic receptor subtypes in the control of intestinal motility. Groups of 4 Beagle dogs were prepared each with a chronically implanted miniature pressure transducer (Konigsberg) in the jejunum. After feeding, the dogs were mildly restrained in a Pavlov sling and intestinal motility and a lead II ECG recorded continually.

Basal digestive motility showed the characteristic pattern of irregular contractile activity which was unaffected by intravenous saline administration. Atropine (10-70 μg kg^{-1} iv) dose dependently inhibited digestive motility with dose levels above 30μg kg^{-1} producing marked tachycardia. In contrast pirenzepine over a dose range (5-50μg kg^{-1} iv) previously shown to be selective at the M_1-subtype (Hirschowitz et al 1983, Schiavone et al 1988), increased digestive motility. At the relatively high dose of 300μg kg^{-1} iv pirenzepine inhibited motility. In contrast to atropine, no effects on heart rate were observed with pirenzepine at any dose level. The M_2 selective antagonist methoctramine at dose levels of 10 and 30μg kg^{-1} iv had no effect on intestinal motility but did evoke a marked tachycardia at the higher dose level. 4-diphenylacetoxy-N-methyl piperidine (4-DAMP), an antagonist with similar affinity for muscarinic receptors of the M_1 and M_3-subtypes but slightly weaker on the M_2-subtype (Lazareno & Roberts 1989), was a potent inhibitor of digestive motility being active over the dose range 1-10μg kg^{-1} iv. This latter agent produced a slight tachycardia at 10μg kg^{-1}.

Assuming tachycardia is a result of muscarinic M_2 antagonism in the heart inhibiting vagal drive then these results suggest that functionally M_2 receptors may be of minor importance in the control of digestive motility in the dog as methoctramine was inactive on the gut at a dose producing marked tachycardia. Results with pirenzepine support the evidence for an inhibitory role of the muscarinic M_1-subtype as suggested by earlier work with the M_1 agonist McN-A-343 (Fox et al 1985, Schiavone et al 1988). The inhibitory effect of high dose pirenzepine is most likely due to loss of selectivity with M_3 antagonism becoming apparent but may suggest a secondary excitatory role for the M_1-subtype. As only inhibition was seen with either the non-selective agent atropine or the M_1/M_3 selective agent 4DAMP, the excitatory component of the muscarinic response would appear to be mediated via M_3 receptors and probably provides the predominant expression of cholinergic activity in the conscious dog. Confirmation of these findings will depend on the identification of agents with a greater selectivity for muscarinic receptor subtypes than those currently available.

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155P EVALUATION OF ICI D7114, A PUTATIVE STIMULANT OF BROWN ADIPOCYTES, ON HISTAMINE PRE-CONTRACTED GUINEA-PIG ILEUM

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Agonists such as BRL 37344 with high affinity for the atypical β-adrenoceptor found on brown adipocytes can elicit inhibition of histamine pre-contracted guinea-pig ileum (Bond & Clarke, 1987; Arch et al., 1984). This effect was insensitive to blockade with propranolol at concentrations greater than those known to completely block either β_1 or β_2 adrenoceptors. The aim of the current study was to investigate the effects of the novel brown adipocyte stimulant, ICI D7114 (Holloway et al., 1991), on the histamine pre-contracted guinea-pig ileum.

Inhibition of histamine-induced contractions of the guinea-pig ileum (Bond & Clarke 1987), were evaluated for effects with isoprenaline, BRL37344 and ICI D7114. In addition, a variety of pharmacological agents was assessed for their ability to modify the response to these agents (Table 1).

Table 1 Potency of ICI D7114, BRL 37344, isoprenaline & the effect of various antagonists.

	D7114	BRL37344	Isoprenaline
Potency (IC_{30} μM)	0.94±0.26	0.25±0.11	0.33±0.05
	Antagonist effects (concentration ratio)		
Methysergide (0.1μM)	2.1 (1.4-2.9)	4.9 (1.2-15.0)	NT
Cimetidine (10μM)	2.8 (0.6-4.6)	4.5 (2.6-8.6)	NT
8-PT (10μM)	1.0 (0.4-1.5)	0.2 (0.03-6.0)	NT
±Propranolol (5μM)	1.6 (1.0-2.3)	12.9 (1.1-32.7)	2.2 (0.2-5.9)
±Cyanopindolol (0.1μM)	4.8 (1.3-7.9)	NT	5.3 (4.7-5.7)

NT = not tested; 8PT =8-phenyltheophylline;

ICI D7114 was similar to the known brown adipocyte stimulant, BRL37344. The concentration ratios obtained in the presence of a range of antagonists suggest that these effects were unlikely to be mediated via β_1 or β_2 adrenoceptors, 5-HT, histamine H_2 or adenosine receptors. It is proposed therefore, that ICI D7114 possesses affinity for the atypical β-adrenoceptor previously identified in the guinea-pig ileum (Bond & Clarke,1987).

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156P RS-87476, A NOVEL NEUROPROTECTIVE AGENT, ANTAGONISES THE EFFECTS OF CALCIUM CHANNEL ACTIVATORS IN GUINEA-PIG TAENIA

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RS-87476 (*piperazine 1-[diphenyl-methyl]-4-[(2-(4-methyl-phenyl)-5-methyl imidazol-4-yl)methyl] trihydrochloride*) has marked neuroprotective activity in models of ischaemic stroke (Alps *et al.*, 1990; Kucharczyk *et al.*, 1991). We have tested the compound for calcium antagonistic activity in a well-characterized smooth muscle preparation: the potassium-depolarized taenia preparation from the caecum of the guinea-pig (Spedding & Berg, 1984; Kenny *et al.*, 1990).

Strips of taenia were set up in 30 ml isolated organ baths under isotonic conditions (see Kenny *et al.*, 1990). The solution had the following composition (mM): NaCl 97, KCl 40, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.5, gassed with 95% O_2/5% CO_2 and maintained at 35 °C.

Cumulative concentration-response curves to calcium (0.01-3 mM) were obtained at 30 min intervals. RS-87476 non-competitively antagonized the responses to calcium with a threshold concentration of 0.1 µM and fully antagonized the responses at 10 µM. In this respect the compound was approximately 100-fold less potent than dihydropyridines like nimodipine.

To assess the effects of RS-87476 on the sensitivity of the preparation to calcium channel activators, the preparations were contracted to 25-45% of their maximum with calcium (0.3-1 mM) and then cumulative concentration-response curves to the calcium channel activators, Bay K 8644 (1-3000 nM) or CGP 28392 (10-10000 nM), were obtained following the protocol described by Kenny *et al.* (1990). Incubation with RS-87476 (10 nM, 30 min) abolished the weak contractile responses to CGP 28392. RS-87476 (10-1000 nM) caused a concentration-dependent inhibition of the responses to Bay K 8644 (see Fig.1); nimodipine is only weakly active under these conditions (Kenny *et al.*, 1990).

Thus, RS-87476 does not act as a conventional calcium antagonist in that responses to calcium channel activators are potently inhibited at concentrations which have little effect on responses to calcium. The converse is found with dihydropyridine calcium antagonists (Kenny *et al.*, 1990). In addition to this novel interaction with calcium channels, RS-87476 has been shown in both functional and ligand binding studies to interact with the voltage-dependent sodium channel (Patmore *et al.*, 1991; Sheridan *et al.*, 1991). Which of these mechanisms is responsible for the neuroprotective activity of RS-87476 has yet to be elucidated.

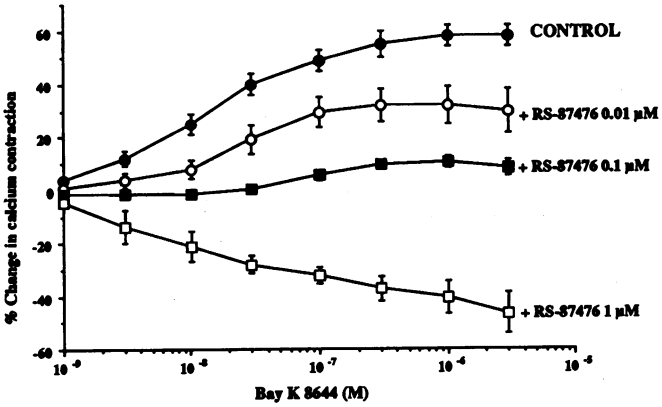

Fig.1 Inhibitory effects of RS-87476 on responses to Bay K 8644 (n = 5-6).

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157P NEURONAL INVOLVEMENT IN CHOLINERGIC REGULATION OF ION TRANSPORT IN RAT COLON

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Cholinergic agonists stimulate ion transport across a variety of intestinal epithelia. Although there is little doubt that functional receptors for acetylcholine exist on epithelial cells, an additional pro-secretory action of cholinomimetics is mediated by nerves within the lamina propria (Kuwahara et al., 1987). We have further investigated neuronal involvement in regulation of ion transport in rat colon *in vitro*.

Sheets of rat colon, stripped of underlying smooth muscle, were mounted in Ussing chambers (window area = 0.63cm^2) and bathed in oxygenated Krebs-Henseleit solution at 37°C. Tissues were voltage clamped by continuous application of short circuit current (SCC). Drugs were added basolaterally to the serosal side bathing solution. Electrical field stimulation (EFS; 6V; 5Hz; 1ms for 1 minute) was applied via silver or aluminium electrodes configured to pass current across tissues. Results are expressed as mean ± s.e.mean.

Carbachol stimulated SCC across isolated colonic mucosae in a concentration-dependent manner (EC_{50} = 7.0±0.7 μM; n=6). Bumetanide (100 μM) reduced SCC responses to carbachol (100 μM) by 81.9±4.9% ($p<0.01$), thus confirming chloride secretion to be involved in the SCC response of rat colon to cholinomimetics (Browning et al.,1977). Complete concentration response curves in test and control preparations showed that SCC responses to high (100 μM) but not low (1 μM) concentrations of carbachol were significantly attenuated by hexamethonium (1 μM; n=6; $p<0.05$) and by tetrodotoxin (TTX; 0.1 μM; n=5; $p<0.01$). These results indicate that carbachol stimulates SCC by a direct and by a nerve-mediated action, each of which is sensitive to atropine (1 μM) which reduced responses to 100 μM carbachol by 93.7±3.7% (n=6; $p<0.001$). Responses to EFS were reduced by TTX (1 μM) and atropine (1 μM) by 68±8% and 39±3% respectively (n=4). In the presence of TTX (0.1 μM), pirenzepine competitively inhibited SCC responses to carbachol (pA2=6.2; n=4).

McN-A-343, an M_1 selective agonist, stimulated SCC (EC_{50} = 12.4±2.2 μM ; n=4) with a maximal response (22.4±5.6 μA; n=7) which was lower than the maximal response to carbachol (64.8±11.2 μA; n=6). TTX (0.1 μM) reduced responses to McN-A-343 (100 μM) by 73.9±3.8% (n=4).

We have shown that cholinergic stimuli induce chloride secretion in rat colon by activating nerves within the lamina propria, in addition to having a direct action on epithelial cells. These findings support previous studies in rat colon (Zimmerman & Binder,1983) and are comparable to the findings of Przyborski et al. (1991) that McN-A-343 activates electrogenic ion transport in rat duodenum by stimulating a postganglionic cholinergic neural pathway.

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158P ENDOTHELIN-1-INDUCED INHIBITION OF SUBMUCOSAL GLAND SECRETION AND EPITHELIAL ALBUMIN TRANSPORT IS MEDIATED PARTLY BY ACTIVATION OF PROTEIN KINASE C

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Endothelin-1 (ET-1) potently contracts vascular and non-vascular smooth muscle including that in the airways. We previously examined the effects of ET-1 on tracheal submucosal gland secretion (lysozyme secretion from serous cells) and epithelial function (active albumin transport), and the role of dihydropyridine-sensitive Ca^{2+} channels in the responses to ET-1. ET-1 had no effect on baseline submucosal gland secretion and epithelial albumin transport but inhibited stimulated (methacholine and phenylephrine) lysozyme output and epithelial albumin transport. The effects of ET-1 were partially mediated through activation of dihydropyridine-sensitive Ca^{2+} channels. Other studies suggest ET-1 may act by releasing prostaglandins or by activation of the membrane-bound enzyme protein kinase C (PKC). In this study we assessed the role of prostaglandin release using indomethacin, and of PKC using H-7 (PKC inhibitor) in the tracheal responses to ET-1.
Air-filled tracheas mounted in organ baths were bathed on the submucosal side with Krebs buffer containing BSA (4mg.ml^{-1}) and fluorescent BSA (40ug.ml^{-1}). Secretions were periodically removed from the trachea for analysis of lysozyme and albumin (fluorescence). All drugs were added to the submucosal buffer.
Methacholine (20μM) and phenylephrine (100μM) produced maintained lysozyme and albumin outputs of 630 ± 63 and 782 ± 69ng.min^{-1}, and 2.92 ± 0.47 and 2.15 ± 0.11μg.min^{-1} respectively.

Table 1. The effect of indomethacin (10uM) and H-7 (5uM) on the inhibition of methacholine and phenylephrine-induced lysozyme and albumin output produced by ET-1 (10uM)

	Methacholine			Phenylephrine		
	Control	Indomethacin	H-7	Control	Indomethacin	H-7
Lysozyme	-44.3±11.6	-52.3±4.7	-28.2±4.9*	-44.0±15.9	-38.7±7.4	-25.3±9.3*
Albumin	-27.9±6.9	-24.2±5.5	-24.6±3.7	-40.2±6.8	-38.9±10.3	-18.1±10.3*

Values shown are % inhibition of methacholine or phenylephrine-induced response by ET-1, and are means ± s.e.mean of six values. *Significantly different from control response to ET-1 by Students paired t-test.

Thus, ET-1-induced inhibition of methacholine and phenylephrine-stimulated serous cell secretion and methacholine-stimulated epithelial albumin transport is mediated in part by activation of PKC. The release of prostaglandins is not involved in the inhibitory effects of ET-1 on serous cell secretion or epithelial albumin transport.

159P CHARACTERISATION OF PURINOCEPTORS IN RAT PAROTID SALIVARY GLANDS

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ATP stimulates amylase release and elevation of intracellular calcium ($[Ca^{2+}_f]_i$) in rat parotid acini (McMillian et al., 1987, 1988; Soltoff et al., 1989). The aim of this study was to identify which subtype(s) of purinoceptors mediate these effects. To this end, amylase release and $[Ca^{2+}_f]_i$ were measured as previously described (Argent & Arkle, 1985; Arkle et al., 1989) in parotid slices and dissociated acini incubated in the presence of purinergic agents and in buffers of varied $MgCl_2$ content.

Whereas ATP was a weak secretagogue, it had a marked, dose-dependent effect on $[Ca^{2+}_f]_i$ (table 1). With the exception of high concentrations of ADP, which slightly increased $[Ca^{2+}_f]_i$ (table 1), other purinergic agents (300μM-adenosine, 100μM-2-methylthioATP and 100μM-β,γ-methyleneATP) were without effect on amylase release and $[Ca^{2+}_f]_i$. Elevation of $[Ca^{2+}_f]_i$ by ATP was not altered by 300μM-adenosine but was more than halved when acini were incubated with ADP or increased $MgCl_2$ (table 1). At concentrations less than 100μM neither reactive blue 2 nor coomassie brilliant blue inhibited ATP-stimulated secretion.

Table 1 Effects of purinergic agents on $[Ca^{2+}_f]_i$ in fura 2-loaded parotid acini (mean ± s.e. mean)

	ATP^{4-} (μM)									
								10mM ADP	10mM ADP + 11.6mM $MgCl_2$	
	0	3	10	30	100	300	1000		100μM-ATP	+ 100μMATP
$[Ca^{2+}_f]_i$ (nM)	222 ±19	221 ±29	297 ±40	442 ±73	539 ±59	822 ±122	413 ±93	312 ±21	326 ±27	356 63

These data lead us to conclude that receptors mediating ATP-stimulated secretion and elevation of $[Ca^{2+}_f]_i$ are not of the P_1-, P_{2x}- or P_{2y}-subtypes. As ATP's effects are inhibited by ADP and by increased $MgCl_2$ content of incubation buffers (which decreases ATP^{4-} activity) it is likely that this receptor is of the P_{2x} subtype.

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160P CONTRASTING PROPERTIES OF BRADYKININ RECEPTOR SUBTYPES MEDIATING CONTRACTIONS OF THE RABBIT AND PIG ISOLATED IRIS SPHINCTER PUPILLAE PREPARATIONS

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In the rabbit iris, bradykinin contracts the sphincter smooth muscle indirectly via the release of neurokinins from sensory nerve fibres within the preparation so has been used to model neurogenic inflammation. In contrast, in the pig preparation bradykinin contracts the sphincter by a direct action on the smooth muscle (Geppetti *et al., 1990*). We have compared bradykinin receptors in these two preparations to gain information on variations between species.

Preparations of sphincter muscle were separated and mounted isometrically in Krebs' solution containing guanethidine [3 μM], mepyramine, atropine, ibuprofen [1 μM] and hexamethonium [10 μM]. Agonists were used to obtain cumulative concentration-response curves, using matched preparations to estimate the effect of previously applied antagonists.

The BK_1 selective agonist [des-Arg^9]-BK was without effect in the rabbit, though there was some activity in certain of the pig preparations. Consistently, where responses to [des-Arg^9]-BK were evident, the BK_1-selective antagonist [des-Arg^9,Leu^8]-BK attenuated these responses though with non-linear Schild regression. A number of bradykinin receptor antagonists were used. In the rabbit preparation the BK_2 receptor antagonist Lys,Lys-[Hyp^3,Thi^{5,8},D-Phe^7]-BK gives pK_B estimates of *ca.* 7.5 (Field *et al*, 1988; Griesbacher & Lembeck, 1987). In the pig preparation, the pK_B for Lys,Lys-[Hyp^3,Thi^{5,8},D-Phe^7]-BK was low (6.0 ± 0.1; n = 11) as compared to typical BK_2 receptor preparations (Field *et al.*, 1988: Hall *et al.*, 1991). The low pK_B estimate was not due to ligand degradation since it did not differ significantly when determined in the presence of peptidase inhibitors (enalaprilat, phosporamidon and mergetpa [1 μM]. In both preparations the novel BK_2/BK_3 antagonist D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (HOE140) was very active, though consistently more potent in the rabbit than the pig.

In summary, the present work demonstrates marked differences in the bradykinin receptors between the rabbit and pig irides. It may be noted that differences have also been reported between the bradykinin receptors in sheep as compared to rabbit irides (Magbagbeola, 1989). In the rabbit, the receptors are located largely on sensory nerve endings, are of the BK_2 subtype, and contract the iris smooth muscle indirectly via release of neurokinins. In contrast, in the pig, evidence favours a direct postjunctional locus of action, and the receptors have properties that do not resemble any one single bradykinin receptor subtype. It may be concluded that extrapolation from any one species to man seems be not without dangers.

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161P COMPARISON OF THE EFFECTS OF K^+ CHANNEL BLOCKERS AND CROMAKALIM IN GUINEA PIG DETRUSOR AND RAT MESENTERIC ARTERY

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K^+ channel openers such as cromakalim (CROM) are of potential benefit in a variety of therapeutic areas including hypertension and urinary incontinence. The aim of the present study therefore, was to compare the K^+ channel pharmacology of vascular and bladder smooth muscle using CROM and a range of relatively selective K^+ channel blockers.

Changes in tension were recorded isometrically from rings of rat mesenteric artery (RMA) and strips of guinea-pig detrusor (GPD) under 1g and 2g resting tension respectively. In RMA studies, the effects of drugs were assessed against either resting tension or against noradrenaline (NA, 0.3uM) induced tone. In GPD, the effects of drugs were evaluated against either resting tension or K^+ (15mM)-evoked average mechanical activity (AMA).

In RMA, Charybdotoxin (ChTX, 0.1μM) had no effect on basal tone but enhanced tone in NA precontracted preparations by 37.8$\pm$14.2% of the NA response. In contrast, ChTX (0.1nM-0.3uM) stimulated basal AMA in GPD in a concentration related manner:maximum response=45.7$\pm$12.0% of K^+ (80mM) (reference maximum) and ChTX (30nM) increased K^+ (15mM)-evoked AMA by 462%. ChTX (0.1uM) failed to antagonise the response to CROM in NA precontracted RMA. In contrast, ChTX (30nM) antagonised the effects of CROM in the K^+ stimulated GPD in a non-competitive manner (depression of maximum to 62.7$\pm$10.3% of control). The potency of CROM was compared in GPD stimulated with either K^+ (15mM) or ChTX (20nM) which stimulated AMA to 99.7% of that evoked by K^+. The potency values for CROM in these studies were not significantly different: EC_{50} v K^+=0.36$\pm$0.09uM; EC_{50} v ChTX=0.31$\pm$0.06uM. In additional studies, Glibenclamide (GLIB) antagonised the effects of CROM in RMA (pA2=6.78$\pm$0.11, slope, 1.2) and in GPD (pA2=6.76$\pm$0.23, slope, 1.35). In both cases the antagonism was apparently competitive. Apamin (0.1uM) was without effect in RMA. In GPD however, apamin increased K^+-evoked AMA by 164.9% and apamin (1-300nM) produced small increases (<10% of K^+ (80mM)) in basal AMA when given non-cumulatively. NTX (0.1nM-1μM) was without effect in both RMA and GPD.

In conclusion, both small (apamin sensitive) and large (ChTX sensitive), Ca^{++}-activated K^+ channels appear to be involved in the regulation of basal tone in GPD but not RMA. Furthermore, K^+ induced tone in GPD appears to be associated with a greater degree of activation of these channels than NA induced tone in RMA. This observation probably explains the functional interaction between CROM and ChTX in GPD. The finding that GLIB antagonised the effects of CROM in both preparations suggests that these effects are mediated by a common ATP-sensitive K^+ channel.

162P THE ROLE OF K^+-CHANNELS IN THE MECHANISM OF ACTION OF ISOPRENALINE: COMPARISON BETWEEEN GUINEA-PIG TRACHEA AND RAT MESENTERIC ARTERY

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β-adrenoceptor activation has recently been shown to be associated with the opening of large conductance, calcium activated potassium channels (BK) in tracheal smooth muscle (Jones et al., 1990). Activation of BK by cAMP has also been demonstrated in vascular smooth muscle (Sadoshima et al., 1988). The purpose of the present study was to investigate whether BK activation was responsible for the effects of β-adrenoceptor activation in the rat mesenteric artery, by studying the interaction between isoprenaline and a range of K^+-channel blockers. Parallel experiments were conducted in the isolated guinea-pig trachea for comparison.

Isometric recordings were made from guinea-pig tracheal chains (GPT) or rings of rat mesenteric artery (RMA) under a resting tension of 1g. Indomethacin (1μM) was present throughout experiments involving GPT. The effects of drugs were assessed against either carbachol (0.27μM), NA (0.3μM) or KCl (80mM) induced tone.

Isoprenaline relaxed carbachol precontracted GPT with a pD_2 value of 8.1 $\pm$ 0.1. Charybdotoxin (ChTX, 0.1μM) enhanced carbachol induced tone by 13.6 $\pm$ 2.4% and antagonised the effects of isoprenaline (pD_2 values were 8.0 $\pm$ 0.01 and 7.2 $\pm$ 0.1 in the presence of vehicle and ChTX respectively). Iberiatoxin (IbTX, 0.03μM) produced quantitatively similar results to ChTX. Glibenclamide (10μM) did not modify carbachol induced tone or isoprenaline induced relaxation. Isoprenaline relaxed NA precontracted RMA (pD_2 value 6.7 $\pm$ 0.2). ChTX (0.1μM) enhanced NA induced tone by 28.1 $\pm$ 6.2%. IbTX (0.03μM) produced quantitatively similar effects. Glibenclamide (10μM) inhibited NA induced tone by 29.4 $\pm$ 4.2% and TEA (10μM) enhanced NA induced tone by 55.2 $\pm$ 25.4%. TEA but not ChTX, IbTX or glibenclamide antagonised the relaxant effects of isoprenaline in this preparation. Apamin (0.1μM) and noxiustoxin (NTX, 1μM) were without effect against either carbachol or NA induced tone or isoprenaline induced relaxation in GPT or RMA. Isoprenaline failed to relax KCl precontracted GPT or RMA.

In conclusion, the effects of β-adrenoceptor activation were consistent with K^+ opening in both the GPT and the RMA. However, whereas β-adrenoceptor activation in the GPT appears to be coupled to BK, β-adrenoceptor activation in RMA does not appear to be coupled to this channel, despite the presence of BK in this preparation. Neither do the effects of isoprenaline appear to be mediated by low conductance calcium activated potassium channels or by ATP or voltage sensitive potassium channels.

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163P COMPARATIVE EFFECTS OF FOUR POTASSIUM CHANNEL OPENERS ON THE RAT ILEUM

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Pinacidil (PIN), lemakalim (LEM, BRL38227), minoxidil sulphate ($MxSO_4$) and diazoxide (DZ) are well documented as potassium channel opening agents, causing relaxation in a variety of smooth muscle preparations (Wilson et al., 1989; Piper and Hollingsworth, 1989). The present study was designed to compare the effects of these agents on responses of the rat ileum longitudinal muscle myenteric plexus (LMMP) preparation, induced either by electrical field stimulation (EFS) or by potassium (KCl).

LMMP preparations derived from the distal ileum of male rats were suspended under 1g tension in Krebs' solution (95% O_2/5% CO_2, 37°C). Isometric contractions were elicited in response to either EFS (0.1Hz, 5msec pulse width, supramaximal voltage) or to KCl (15-20mM giving responses 60-75% E_{max}). When reproducible twitches were obtained to EFS, or at the plateau of the KCl induced contraction, PIN (0.1-13μM), LEM (50nM-1.6μM), $MxSO_4$ (1-64μM), DZ (1-64μM) or appropriate vehicle was applied cumulatively. Effects were expressed as percentage reversal of the initial contraction or twitch.

PIN and LEM completely abolished EFS-evoked twitch responses: the IC_{50} values were 1.17±0.16μM (n = 6) and 0.10±0.02μM (n = 6) respectively. $MxSO_4$, at the highest concentration used, caused only a 30% reduction of twitch height. The inhibitory effect of DZ was indistinguishable from the effect of its vehicle (n = 4). Glibenclamide (300nM) significantly reversed the inhibition of twitch response caused by PIN and LEM (n = 4 for each agent) by approximately 75 and 90% respectively. However, glibenclamide produced only a very small reversal of the inhibition of twitch response caused by $MxSO_4$ and did not reverse any apparent effect of DZ or any vehicle effect.

PIN and LEM caused complete concentration dependent relaxations of KCl-induced contractions: the apparent IC_{50} values were 0.65±0.07μM (n = 24) and 0.16±0.02μM (n = 10) respectively. The relaxant effects of DZ were again indistinguishable from those of its vehicle (n = 5). $MxSO_4$ appeared to cause complete concentration dependent relaxations, however, this was associated with a vehicle effect of 52% relaxation at higher concentrations.

These results indicate similarities in effect of PIN and LEM with LEM being more potent. This study also indicates that neither compound preferentially inhibits either direct smooth muscle or nerve mediated responses. The present study showed low potency of $MxSO_4$ and apparent absence of effect of DZ in both experimental protocols contrasting with results from other tissues (Edwards & Weston, 1990) although $MxSO_4$ has been found to exhibit low potency in uterine tissue (Piper and Hollingsworth, 1989).

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164P CROSS-TOLERANCE BETWEEN SALBUTAMOL AND CROMAKALIM IN THE RAT UTERUS *IN VIVO*

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Uterine tolerance to the K-channel opener, cromakalim occurs in vivo and in vitro (Downing et al., 1989; Piper et al., 1991). β-adrenoceptor agonists exhibit cAMP-regulated K-channel opening (Longmore et al., 1991). Cross-tolerance between salbutamol and cromakalim was therefore investigated in the uterus in vivo.

Rats were bilaterally ovariectomized and given an intra-uterine balloon and jugular vein cannula. Dose response curves to the uterine effects of salbutamol and cromakalim were determined (Downing et al., 1989) before and after they received i.v. salbutamol (120μg kg^{-1} h^{-1}) or vehicle for 24 h, propranolol (400μg kg^{-1} h^{-1}) for 2 h or cromakalim (3 x 1 mg kg^{-1} 8 h apart) or vehicle. Pre- and post-treatment log ID_{50} values were determined for each rat. Uterine cAMP concentrations were determined ex vivo after i.v. salbutamol (100 μg kg^{-1}) or cromakalim (0.25 mg kg^{-1}). Uterine sensitivity to salbutamol increased in vehicle-treated and cromakalim-tolerant rats to a similar extent (log ID_{50} values (μg kg^{-1}): pre-vehicle, 1.94±0.16, post-vehicle, 1.28±0.10; pre-cromakalim 2.18±0.18; post-cromakalim 1.44±0.14). Uterine sensitivity to cromakalim increased in vehicle infused rats (log ID_{50} values (mg kg^{-1}): pre-vehicle, -0.76±0.10; post-vehicle, -1.70±0.09). In salbutamol-tolerant rats, maximum achievable uterine response to cromakalim was less than 50% inhibition. Uterine sensitivity to cromakalim was increased in propranolol-infused rats (log ID_{50} values (mg kg^{-1}): pre-propranolol -0.81±0.09; post-propranolol -1.15±0.10). Salbutamol produced a 11.7-fold increase in uterine cAMP concentrations at 2 min whereas cromakalim did not significantly change uterine cAMP concentrations (vehicle, 8.7±1.1; salbutamol, 101.9 ± 20.9; cromakalim, 11.6±2.6 pM cAMP mg protein^{-1}).

Reduced uterine responses to cromakalim in salbutamol-tolerant rats suggests some common mechanism to their action. Lack of propranolol antagonism of cromakalim or sustained cromakalim-induced changes in cAMP concentrations suggests that β-adrenoceptor or adenylate cyclase activation are not common between cromakalim and salbutamol. K-channel opening may be the common mechanism.

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165P POTASSIUM CHANNEL BLOCKING ACTIVITY OF DEQUALINIUM ANALOGUES IN GUINEA-PIG HEPATOCYTES AND RAT SYMPATHETIC NEURONES

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The small conductance Ca^{2+} activated K^+ channel (SK_{Ca}) channel is selectively blocked by the bee venom toxin apamin. Other blockers include bisquaternary ammonium compounds such as dequalinium (Castle, 1987), tubocurarine and atracurium (Cook & Haylett, 1985). To study the structural requirements for this action, we have synthesized a number of dequalinium analogues, and tested their ability to block SK_{Ca} channels in hepatocytes and sympathetic neurones.

Guinea-pig hepatocytes were isolated, and K^+ loss through SK_{Ca} channels (opened by the increase in $[Ca^{2+}]_i$ caused by angiotensin II) was measured using K^+ sensitive electrodes, as described by Cook & Haylett (1985). The new compounds were tested for their ability to block this response, as well as the SK_{Ca} mediated slow after-hyperpolarization which follows the action potential in sympathetic neurones. These neurones were dissociated from superior cervical ganglia of 17 day old rats and maintained in culture for 7 to 10 days as described by Smart (1987). Intracellular recordings were made using conventional microelectrodes, and action potentials were evoked every 5s by injection of depolarizing current. Blocking potency was measured as the concentration of drug required to reduce the K^+ loss or after-hyperpolarization by 50% (IC_{50}). The results are shown in Table 1.

Compound	IC_{50} Hepatocyte (μM)	IC_{50} Neurone (μM)
1,1'-decamethylenebis[4-amino-2-methylquinolinium] (dequalinium)	3.7 ± 0.5	1.0 ± 0.1
1,1'-decamethylenebis[4-aminoquinolinium] (UCL1127)	7.5 ± 1.0	1.6 ± 0.1
1,1'-decamethylenebisquinolinium (UCL1072)	16.0 ± 2.0	21.0 ± 2.0
1,1'-decamethylenebis[4-aminopyridinium] (UCL1054)	13.0 ± 1.0	35.0 ± 2.0
10,10'-decamethylenebis[9-amino-1,2,3,4-tetrahydroacridinium] (UCL1130)	2.2 ± 0.1	1.0 ± 0.1
1-decyl-4-amino-2-methylquinolinium (UCL1092)	>>30.0	5.5 ± 0.6*

Values are mean ± s.e.mean from 3 to 6 experiments. * indirect effect due to inhibition of Na^+ and Ca^{2+} currents

From these results, the presence of two quaternary aromatic groups with an appropriate separation appears to be important for the potent and selective SK_{Ca} channel blocking activity of dequalinium. The presence of a 2-methyl group, or extending it to a saturated ring, does not appear to be critical to activity. However reducing the size of the aromatic group, or deletion of the 4-amino group, leads to a considerable loss of activity, while the removal of one complete aromatic group with its quaternary nitrogen gives a compound (UCL 1092) with Na^+ and Ca^{2+} channel blocking activity. Though there are some differences in the relative potencies observed with the two assay systems, the evidence taken as a whole suggests that the SK_{Ca} channels in hepatocyte and ganglion cells are not dissimilar.

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166P NPC 15437, A SELECTIVE INHIBITOR OF PROTEIN KINASE C, INHIBITS T CELL ACTIVATION *IN VITRO* AND *IN VIVO*.

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We have recently reported that 2,6,diamino-N-([1-(oxotridecyl)-2-piperidinyl]methyl)-hexanamide (NPC 15437) is a selective inhibitor of protein kinase C (PKC) interacting at the regulatory domain of the enzyme (Sullivan *et al.*, 1991). PKC activity and binding of phorbol ester to the enzyme were inhibited by NPC 15437 with IC_{50} values of 19 ± 2 μM and 23 ± 4 μM, respectively. Significantly, no inhibition of cAMP-dependent or calcium/calmodulin-dependent protein kinases was observed at concentrations of NPC 15437 up to 300 μM.

The sustained activation of PKC has emerged as an important signal transduction mechanism for T cells in response to mitogens such as phytohemagglutinin (PHA) (Berry and Nishizuka, 1990). To investigate the ability of NPC 15437 to penetrate cell membranes and exert biological effects, we have examined the effect of NPC 15437 on human T-cell proliferation and IL-2 mRNA and protein production. The ability of NPC 15437 to inhibit PHA-stimulated T cell proliferation was determined in peripheral blood lymphocytes (PBML) as described (Atluru *et al.*, 1990). PHA (10 μg/ml) and NPC 15437 were added directly to the wells upon initiation of culture. At 24 or 48 hours, NPC 15437 was removed and replaced with fresh media containing PHA. [^{3}H]thymidine (0.5 μCi/well) was added to the cultures 20 hours before harvesting. NPC 15437 inhibited PHA-induced lymphocyte proliferation with an IC_{50} of 4 ± 2 μM. No change in the IC_{50} was observed when NPC 15437 was removed after either 24 hours or 48 hours. This is not unexpected since the involvement of PKC in the activation process occurs between 0 and 8 hours (Berry and Nishizuka, 1990). Cell viability, assessed by determining lactate dehydrogenase activity in the media of cells incubated with NPC 15437 for 72 hours, was not affected by concentrations of inhibitor up to 30 μM. IL-2 production was measured using a cytotoxic lymphoid cell line (CTLL-2). NPC 15437 concentration-dependently antagonized the production of IL-2 with an IC_{50} value of 3 ± 1 μM. Addition of exogenous IL-2 reversed the inhibition caused by NPC 15437. Northern blot analysis of PHA-stimulated T cells indicated that NPC 15437 blocked induction of IL-2 gene expression at concentrations that had no effect on cell viability. Finally, oxazolone applied to the ears of sensitized mice causes a type IV hypersensitivity response, maximal at 24-48 hours. NPC 15437 applied topically in ethanol a single time 30 minutes prior to oxazolone, inhibited edema with an IC_{50} value of 11 μg/ear.

These results demonstrate that NPC 15437 can readily penetrate cell membranes and block the critical, early events involved in T cell activation. Further the compound possesses anti-inflammatory activity *in vivo*, inhibiting the dermatitis elicited by oxazolone. Thus, the actions of NPC 15437 are consistent with the postulated involvement of PKC in a variety of inflammatory processes.

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167P THE ACTIVITY OF OCTIMIBATE AT PROSTANOID RECEPTORS ON HUMAN MONOCYTES

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Octimibate is an antihyperlipidaemic, antiartherosclerotic drug with antithrombotic properties (Lautenschlager *et al.*, 1986). Inhibition of platelet aggregation by octimibate seems to occur by activation of prostacyclin receptors, although octimibate appears to be only a partial agonist for stimulation of adenylyl cyclase in human platelet membranes (Merritt *et al.*, 1991). In this study we have investigated the effect of octimibate on the responses of a human monocyte cell line, Mono Mac 6.

Mono Mac 6 cells were grown in RPMI 1640 medium supplemented as described (Ziegler-Heitbrock *et al.*, 1988) and plated out at 2.5 x 10^6 cells/ml into 18 well trays for determination of TNFα production (assayed by ELISA) in response to 24h incubation with 100ng/ml lipopolysaccharide (LPS). Stimulation of adenylyl cyclase in Mono Mac 6 cells was measured by the production of [^{3}H]-cyclic AMP from cells preloaded with [^{3}H]-adenine (Barber *et al.*, 1980). Mono Mac 6 cells (2.5 x 10^5 cells/200μl) were incubated for 30min at 37°C in RPMI 1640 containing 100μM-rolipram, 3μM-indomethacin, and 2U/ml adenosine deaminase followed by a further 10min with test compound. The reaction was stopped by the addition of ice-cold TCA and total [^{3}H]-cyclic AMP determined.

Iloprost (a stable prostacyclin analogue), PGE_2 and NECA all produced a concentration-dependent increase in [^{3}H]-cyclic AMP production by Mono Mac 6 cells with half-maximally effective doses of 1.67 +/- 0.50, 0.054 +/- 0.003, and 3.70 +/- 0.71μM respectively (mean +/- s.e.mean, n=3). Octimibate failed to stimulate adenylyl cyclase even at 10μM. However when concentration-response curves to iloprost, PGE_2 and NECA were performed in the presence of 10μM-octimibate, the curves to both iloprost and PGE_2 were clearly shifted to the right in a parallel fashion whereas the response to NECA was unaffected. EC_{50} values in the presence of 10μM-octimibate were 1.082 +/- 0.248 and 3.44 +/- 0.83μM for PGE_2 and NECA respectively (mean +/- s.e.mean, n=3). The EC_{50} value for iloprost in the presence of 10μM-octimibate was estimated at approximately 140μM (n=3).

Mono Mac 6 cells were incubated with iloprost, PGE_2, and NECA (+/- 10μM-octimibate) at concentrations shown to inhibit LPS-stimulated TNFα secretion by 50%. Octimibate antagonised the effect of both 30nM-iloprost and 3nM-PGE_2 but had no effect on the ability of 30μM-NECA to inhibit TNFα secretion.

Octimibate therefore displays highly tissue-specific responses and behaves as an antagonist, rather than an agonist, at prostacyclin receptors on a human monocyte cell line. Octimibate appears to be selective for prostanoid-type receptors being able also to inhibit PGE_2 but not adenosine A2 receptors on Mono Mac 6 cells.

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168P THE ANTI-INFLAMMATORY ACTIONS OF MANSUMBINOIC ACID AND MANSUMBINONE, TWO NOVEL TRITERPENES FROM *COMMIPHORA INCISA*

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Following preliminary observations of anti-inflammatory activity shown by various extracts from the resin of *C.incisa*, two novel triterpenes of the dammarane type isolated from that species (Provan & Waterman 1986) were tested for anti-inflammatory activity using the carrageenan-induced paw oedema model of acute inflammation. Male Sprague Dawley rats were pre-dosed with suspensions (5% Tween 80, saline) of the compounds (p.o.) two hours prior to the induction of oedema (Winter *et al* 1962). Oedema was monitored as percent increase in paw diameter. Indomethacin and Prednisolone were used as positive controls.

Table 1: Effect of mansumbinone and mansumbinoic acid on carrageenan-induced oedema

Drug	dose moles/Kg	% suppression of peak of oedema	% suppression Mean area under time-course curve
mansumbinone	1.27x10-4	12.6	20.0*
	2.5x10-4	12.6	40.0*
mansumbinoic acid	8x10-5	30.0*	31.5*
	1.27x10-4	57.1*	57.2*
indomethacin	0.7x10-5	40.0*	33.6*
	1.4x10-5	50.0*	52.0*
prednisolone	0.7x10-5	33.6*	31.9*
	1.4x10-5	55.0*	55.8*

Significant differences between the drug and control groups are indicated as * < 0.05 (Mann Whitney U test).

Mansumbinoic acid, being more active, was tested on adjuvant-induced arthritis in male wistar rats. It significantly inhibited the polyarthritis phase in the paw by 43.0% and 28.9% ($p < 0.05$) in the tibio-tarsal joint.
We conclude that mansumbinoic acid is the more active of the two triterpenes tested and that the anti-inflammatory activity of the crude resin is probably due mainly to these two compounds because of their relative abundance in the resin. Whereas mansumbinoic acid is about 8 times less potent than either prednisolone or indomethacin, mansumbinone is over 10 times less potent.

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169P INTERLEUKIN-1α INHIBITS THE BINDING OF PROSTAGLANDIN E_2 TO MACROPHAGES/MONOCYTES

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The monocyte/macrophage participates in a variety of immune functions including production of immunologically active mediators such as interleukin-1 (IL-1). IL-1 is directly involved in the activation of helper T lymphocytes, and the stimulation of other cell types including monocytes/macrophage to produce secondary mediators such as PGE_2 (Dinarello, 1985). PGE_2 is known to inhibit various immune responses possibly by raising the intracellular cyclic AMP (cAMP) level through the activation of receptor-linked adenylate cyclase in the monocyte/macrophage and other target cells. Inhibition of IL-1 production in monocytes by PGE_2 is an event fundamental to the regulation of immune responses. During monocyte/macrophage activation, PGE_2 is thought to act as a feedback regulator in normal immune responses. But little is known as to how PGE_2 and IL-1 receptors are involved in regulating the behaviour of monocytes during immunoactivation. Here we report that the preincubation of $P388D_1$ cells with increasing concentrations of IL-1 inhibits the binding of $[^3H]PGE_2$.

Murine macrophage-like cells, $P388D_1$, were grown in suspension, harvested and washed twice with RPMI 1640. The cells ($2x10^6$) were then preincubated for 30 minutes with various concentrations of human recombinant IL-1α (0-73 pM) at 21°C before the addition of $[^3H]$ PGE_2 (3.0 nM,) and further incubated for 20 min at 4°C. After incubation, the cells were vacuum filtered through GF/C filters. Filters were washed with 10 ml ice-cold phosphate-buffered saline and radioactivity was measured by scintillation counting. Non-specific PGE_2 binding was estimated by determining the radioactivity bound to filter paper in the presence of a 1000-fold excess of unlabelled PGE_2. Cyclic AMP was estimated by radioimmunoassay.

Preincubation of cells with IL-1α decreased the $[^3H]PGE_2$ binding in a concentration-dependent manner. IL-1α at 58 pM inhibited the specific PGE_2 binding to these cells by almost 90% whereas heat-denatured IL-1α had no effect on the $[^3H]PGE_2$ binding. Scatchard analysis of $[^3H]PGE_2$ binding to the cells shows that the preincubation of IL-1α (29.8 pM) decreased both the high (0.30 fmol/10^6 cells to 0.12 fmol/10^6cells) and low affinity (from 2.40 fmol/10^6 cells to 1.51 fmol/10^6 cells) binding capacities without changing their affinities (Kd_1=2.27 nM, Kd_2=40 nM for the high and low affinity binding sites respectively). IL-1α at 29.8 pM reduced the total PGE_2 binding from 0.325 fmol/10^6 cells to 0.173 fmol/10^6 cells. The observed total binding both in the absence and presence of various concentrations of IL-1α was found to be within the 5-10% of the estimated theoretical calculation of total binding using the following equation (Klotz & Huntson, 1971); total binding = $n_1[L]/(Kd_1+[L]) + n_2([L]/(Kd_2+[L])$, where L is the ligand concentration and n the number of binding sites. Cyclic AMP levels in response to PGE_2 (3 nM) increased from 423 ± 38 to 633 ± 25, with IL-1α (150 pM) the level was 493 ± 28 and in the presence of both PGE_2 and IL-1α the level was 565 ± 30 (all n = 4 ± s.d. of fmol/ 10^6 cells).

These data indicate that IL-1 may exert a feedback control by preconditioning monocytes to bind less PGE_2 in order to minimise the immunosuppressive action of this prostanoid. Measurement of cyclic AMP levels in $P388D_1$ cells suggest that down-regulation of PGE_2 receptors by IL-1 is not mediated through the modulation of cyclic AMP levels.

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170P EFFECT OF PF 5901 ON PAF-INDUCED AIRWAY RESPONSES IN NEONATALLY IMMUNISED RABBITS

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PAF has previously been demonstrated to induce bronchial hyperresponsiveness (BHR) in some, but not all, normal rabbits (Coyle *et al.*, 1990*a*). We have however, recently shown that administration of PAF to neonatally immunised rabbits will induce long-lasting BHR in all animals (Herd & Page, 1991), although the mechanism contributing to this BHR has not been elucidated. In the present study, NZW rabbits were immunised neonatally i.p. with *Alternaria tenius* in $Al(OH)_3$ gel. This procedure was repeated weekly for one month, then biweekly for the next 2 months, as previously described (Coyle *et al.*, 1990*b*). Airway hyperresponsiveness to aerosolised histamine was assessed 24 hr prior to, and 24 hr, 72 hr and 1 week following the administration of PAF (80 μg/ml via aerosol for 1 hr). The 5-lipoxygenase inhibitor PF 5901 (10 mg, 3 mg or vehicle) was administered in a 0.5 ml suspension of PEG 400 and saline instilled directly into the lungs of rabbits 1 hr prior to the PAF challenge. The concentration of histamine required to elicit a 50% increase in airways resistance (R_L) (PC_{50}) and a 35% drop in dynamic compliance (C_{dyn}) (PC_{35}) was calculated. Pretreatment with PF 5901 (10 mg) inhibited the bronchoconstriction induced by PAF. PF 5901 (3 and 10 mg) significantly inhibited the development of BHR (both R_L and C_{dyn}) at any of the measured time points (see Table 1) ($p<0.05$, * = significant compared with PRE value).

Table 1 p

	PC_{50} (mg/ml) (mean)				PC_{35} (mg/ml) (mean)			
	PRE	24 hr	72 hr	1 week	PRE	24 hr	72 hr	1 week
no treatment (n=7)	18.16	2.85*	2.80*	16.79	16.26	4.21*	4.51*	4.80*
vehicle (n=3)	17.22	4.26*	5.72*	3.62*	9.13	4.09*	6.18*	4.88*
PF 5901 (10mg) (n=6)	11.54	11.51	11.32	8.00	7.83	8.57	6.98	3.87
PF 5901 (3mg) (n=3)	15.10	23.88	32.89	11.37	9.30	20.48	11.89	7.13

These results indicate that 5-lipoxygenase products may contribute both to PAF-induced bronchoconstriction and to the persistent hyperresponsiveness induced by PAF in immunised rabbits.

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171P NEONATAL IMMUNIZATION OF RABBITS USING HOUSE-DUST MITE ALLERGEN (Der p I) INDUCES AIRWAY HYPERRESPONSIVENESS

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A characteristic feature of the asthmatic individual is the presence of bronchial hyperresponsiveness. Previous studies have demonstrated that neonatal immunization of rabbits with Alternaria tenius leads to a model of persistent bronchial hyperresponsiveness (McKenniff et al., 1991). House-dust mite (HDM) allergens have been implicated as a major source of atopic sensitization in man. Furthermore, it has been suggested that exposure in early childhood to HDM allergens is an important determinant in the subsequent development of asthma (Sporik et al., 1990). Therefore our aim was to devise a unique model of neonatal HDM immunization to investigate the early events leading to allergic bronchial hyperresponsiveness and asthma.

NZW rabbits were immunized within 24 hours of birth with either HDM allergen in aluminium hydroxide ($Al(OH)_3$) gel or HDM allergen alone. This procedure was repeated weekly for the first month and then biweekly for the following two months, as previously described (Coyle et al., 1990). At 13 weeks the airway responsiveness to aerosolized histamine was assessed, and the dose of histamine required to elicit a 50% increase in airway resistance (PD_{50}) and a 35% fall in dynamic compliance (PD_{35}) was calculated.

	HDM+ $Al(OH)_3$	HDM	saline	naive
PD_{50} mean	8.7	8.0	29.4	37.5
(mg/ml) s.e.mean	(5.6-13.5)	(5.3-12.2)	(18.7-46.1)	(25.4-55.5)
PD_{35} mean	12.0	11.4	29.8	41.6
(mg/ml) s.e.mean	(8.5-16.9)	(9.3-14.0)	(19.4-45.7)	(30.1-57.4)
	(n = 4)	(n = 4)	(n = 7)	(n = 7)

These results suggest immunization with either HDM and adjuvant or HDM alone promotes hyperresponsiveness of the airways when compared to naive and saline-immunized controls ($P<0.05$). No difference in direct skin test sensitivity to allergen was observed between the HDM-immunized groups (range 1:100 - 1:1000). Total cell counts recovered in lavage fluid were similar in all groups, however there was a significant neutrophillic infiltration in treated compared to naive rabbits ($P<0.05$). Neonatal immunization with HDM, in the presence and absence of adjuvant, promotes bronchial hyperresponsiveness.

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172P EFFECT OF FLOSEQUINAN AND BTS 53 554 UPON ISOENZYMES OF PHOSPHODIESTERASE (PDE) ISOLATED FROM GUINEA-PIG VENTRICLE USING MONO Q FPLC

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Flosequinan is currently under clinical trial for the treatment of heart failure. We have previously reported that flosequinan and its major metabolite BTS 53 554 (7-fluoro-1-methyl-3-methylsulphonyl-4-quinolone) are weak, non-selective inhibitors of PDE isoenzymes I,II and III isolated from guinea-pig ventricle using diethylaminoethyl cellulose chromatography (DEAE) (Frodsham et al., 1990). Using this technique it is possible that the rolipram sensitive (PDE IV) isoenzyme may have co-chromatographed with another isoenzyme type thereby influencing the inhibition profile observed.

The use of fast protein liquid chromatography (FPLC) with a Mono Q column produced a very similar elution profile to that obtained using DEAE but resolved an additional minor peak next to the previous PDE II peak. The isoenzymes, characterised using substrate kinetics, regulatory properties and inhibition by standard compounds, have been used to assess the profile of inhibition of flosequinan and BTS 53 554. PDE activity was measured by the method of Thompson et al. (1979). PDE inhibition was observed using 1μM cGMP as substrate for PDE's I and II and 1μM cAMP for PDE's III and IV.

Using FPLC separation, PDE isoenzymes I, II and III were resolved together with an additional minor peak which was identified as PDE IV (IC_{50} of 0.84μM for inhibition by rolipram). The selectivity ratios (SR:IC_{50} PDE I (cGMP)/IC_{50} PDE III (cAMP)) for isobutylmethylxanthine (IBMX), zaprinast, and milrinone were 1.4, 0.009 and 85.7 respectively, compared with 1.5, 0.005, and 68.4 previously reported using DEAE chromatography. Flosequinan and BTS 53 554 were weak inhibitors of PDE I (IC_{50}'s 2.15 and >3.00 mM) and PDE IV (1.75 and >3.00 mM) respectively. Although slightly more potent as inhibitors of PDE II (IC_{50}'s 0.50 and 0.63 mM) and PDE III (0.54 and 0.87 mM) respectively, the calculated SR's were 4.0 and >3.4 (FPLC) compared to 3.6 and >3.0 (DEAE) respectively, and are indicative of non-selective PDE inhibition.

We conclude that flosequinan and BTS 53 554 are not PDE IV selective inhibitors and therefore confirm the weak non-selective inhibitory activity demonstrated previously.

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173P EFFECT OF FLOSEQUINAN ON cGMP AND cAMP LEVELS IN ISCHAEMIC AND NON-ISCHAEMIC RAT VENTRICLE

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We have previously shown that flosequinan has no effect on ischaemic arrhythmias in anaesthetised rats (Jones et al., 1990). In this study we have determined the effect of flosequinan, milrinone and isoprenaline on ventricular cAMP and cGMP levels in anaesthetised rats following coronary artery ligation.

Male Wistar rats (Olac UK) 300-400g were anaesthetised with pentobarbitone and coronary artery ligation performed by the method of Clark et al. (1980). Drugs, at doses causing approximately 50% fall in mean blood pressure, or saline were given intravenously and the coronary ligature tied 5 min later. Hearts were either removed 15 minutes following coronary ligation, at a time of peak arrhythmogenesis, or after 30 seconds of sustained ventricular fibrillation. The ischaemic left ventricle (LV) was separated from the non-ischaemic right ventricle (RV), freeze clamped and stored in liquid nitrogen until assayed. cAMP and cGMP levels (pmol/mg protein) were determined by radioimmunoassay (Amersham). Values are mean ± s.e. mean.

Table 1 Effect of various treatments on cAMP and cGMP in ischaemic (LV) and non-ischaemic (RV) tissue.

		LV cAMP	RV cAMP	LV cGMP	RV cGMP
Flosequinan 6 mg/kg iv	(n=11)	7.00 ± 0.41	6.87 ± 0.41	1.47 ± 0.19	1.28 ± 0.13
Saline 1ml/kg iv	(n=13)	6.70 ± 0.31	6.34 ± 0.22	1.37 ± 0.10	1.39 ± 0.20
Milrinone 0.3 mg/kg iv	(n=12)	8.44 ± 0.55*	8.51 ± 0.33	0.38 ± 0.03	0.36 ± 0.03
Saline 1 ml/kg iv	(n=12)	6.94 ± 0.27	7.62 ± 0.34	0.39 ± 0.06	0.35 ± 0.05
Isoprenaline 0.1 µg/kg/min iv	(n=12)	8.54 ± 0.39	9.01 ± 0.55*	2.81 ± 0.28	2.15 ± 0.19
Saline 0.3ml/kg/min iv	(n=11)	7.57 ± 0.53	7.36 ± 0.39	2.60 ± 0.19	2.08 ± 0.11

None of the treatments significantly altered cGMP levels in ischaemic or non-ischaemic ventricular tissue. Flosequinan was without effect on cAMP levels whereas both milrinone and isoprenaline significantly elevated cAMP levels in LV or RV tissue respectively (* $P < 0.05$ two sample t test). We conclude that flosequinan has no effect on cyclic nucleotide levels in ischaemic or non-ischaemic ventricular tissue in anaesthetised rats at a dose causing substantial hypotension.

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174P FLOSEQUINAN AND ITS MAJOR METABOLITE, BTS 53 554, DO NOT INTERACT WITH THE L-TYPE CALCIUM CHANNEL FROM GUINEA-PIG VENTRICLE

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Flosequinan is currently under clinical trial for the treatment of heart failure. Recent work shows that flosequinan is capable of reducing intracellular calcium levels (Resnick et al., 1991). We have investigated the effect of flosequinan and its metabolite, BTS 53 554 (7-fluoro-1-methyl-3-methylsulphonyl-4-quinolone), on binding of tritiated ligands to two of the known binding sites on the L-type calcium channel to determine if any part of this reduction in intracellular calcium is due to calcium channel blockade.

Crude microsomes were prepared from guinea-pig ventricle using Polytron homogenisation followed by centrifugation at 35,000xg for 1 hour. Binding to the dihydropyridine binding site of the L-type calcium channel using the ligand [^{3}H]-PN200-110 was measured using an assay based on the procedure published by Gould et al. (1984). Binding to the phenylalkylamine binding site was measured using the ligand [^{3}H]-desmethoxyverapamil by the procedure of Garcia et al. (1984). The ligand [^{3}H]-PN200-110 bound to the microsomes with a Kd value of 0.076nM and a Bmax of 0.18 pmoles bound/mg protein. Nifedipine could completely displace [^{3}H]-PN200-110 binding (0.06nM) with an IC_{50} value of 4.9 ± 1.0nM (s.e. mean) for 3 separate experiments. (±)Diltiazem and (±)verapamil could only partially displace this binding at concentrations of up to 10µM (40% and 55% respectively). The ligand [^{3}H]-desmethoxyverapamil bound to the microsomes with a Kd value of 3.1nM and a Bmax value of 0.45 pmoles bound/mg protein. (±)Verapamil could completely displace this binding at 2nM [^{3}H]-ligand with an IC_{50} value of 97 ± 13nM (s.e. mean) for 3 separate experiments. The other ligands, nifedipine and (±)diltiazem, could only partially displace this binding at concentrations of up to 10µM (60% and 80% respectively).

Neither flosequinan nor BTS 53 554 showed any displacement of the binding of these two [^{3}H]-ligands when used at concentrations of up to $1x10^{-4}$M and it is concluded that blockage of the L-type calcium channel would appear not to be involved in the mode of action of flosequinan and its metabolite BTS 53 554 under these conditions.

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175P RS-87476, A NOVEL NEUROPROTECTIVE AGENT, INHIBITS VERATRINE-INDUCED CALCIUM OVERLOAD IN EMBRYONIC CHICK CARDIAC MYOCYTES

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Calcium overload can be induced in cells by procedures which lead to elevation of intracellular sodium levels. These include transient removal of extracellular calcium, treatment with veratrine or exposure to ouabain (Patmore *et al.*, 1989). We have previously shown that the calcium channel antagonists, nicardipine and flunarizine, protect against veratrine-induced calcium overload in cardiac myocytes (Patmore *et al.*, 1989) and that this property correlates with the ability of these compounds to protect against ischaemic neuronal damage in the hippocampus of the Mongolian gerbil (Alps *et al.*, 1988). We now report the effects of RS-87476 (*piperazine 1-[diphenyl-methyl]-4-[(2-(4-methyl-phenyl)-5-methyl imidazol-4-yl)methyl] trihydrochloride*) in the veratrine-induced calcium overload model; this compound has potent neuroprotective actions in a number of animal stroke models (Alps *et al.*, 1990; Kucharczyk *et al.*, 1991).

Calcium overload contractures were induced in aggregates of embryonic chick myocytes by application of veratrine (100 µg ml^{-1}, 5 min) (for details of methods see Patmore *et al.*, 1989). Responses to veratrine were examined in the absence and presence of RS-87476 (10 min pretreatment) and compared using analysis of variance followed by Dunnett's *t*-test. Results are presented in the Table. Previously published data for flunarizine and nimodipine are included for comparison (Patmore *et al.*, 1989).

EFFECT OF COMPOUNDS ON VERATRINE-INDUCED CONTRACTURES

	RELATIVE CONTRACTURE†					
compound	*control*	*n*	*+ compound*	*n*	*% control*	*IC_{50} (µM)*
RS-87476 (5 µM)	2.90 ± 0.27	*(13)*	0.42 ± 0.26 **	*(4)*	14.6	1.6
flunarizine (5 µM)	3.37 ± 0.59	*(4)*	0.70 ± 0.13 **	*(4)*	20.7	2.3
nimodipine (5 µM)	3.31 ± 0.51	*(7)*	3.19 ± 0.47 ns	*(4)*	96.4	-

† *Contractures are expressed relative to the twitch magnitude before drug treatment.* ** $P < 0.01$ ns - $P > 0.05$

The present results demonstrate that RS-87476 potently inhibits veratrine-induced contractures in embryonic chick cardiac myocytes. It appears that compounds effective in this model act by inhibiting the sodium entry which precedes calcium overload (Patmore *et al.*, 1989). Furthermore, RS-87476 inhibits sodium currents in neuronal cells (Sheridan *et al.*, this meeting) and inhibits [^3H]-batrachotoxin binding to rat cortical membranes (IC_{50} = 119 nM). However, an interaction at calcium channels has also been demonstrated (Fraser & Spedding, this meeting).

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176P SUBCUTANEOUSLY ADMINISTERED MDL 73404: ITS DISTRIBUTION AND EFFECTS ON INFARCT SIZE

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It has been previously been reported that MDL 73404 (3,4-dihydro-6-hydroxy N,N,N,2,5,7,8, heptamethyl-2H-1-benzopyran-2-ethanaminium-4-methyl benzene sulfonate), an α-tocopherol analogue, is a free radical scavenger (Bolkenius et al., 1991) that reduces infarct size upon intravenous (iv) infusion in rats subjected to coronary artery ligation and reperfusion (Petty et al., 1991). The compound has now been evaluated for its effects on infarct size produced by coronary artery ligation (60 min) and reperfusion (30 min), following subcutaneous (sc) injection. Administration of MDL 73404 (3 mg/kg) had no effect on infarct size, when administered 4 h before reperfusion. However, when the same dose (3 mg/kg) was administered sc 1 h before reperfusion the infarct size was significantly reduced, being 159 ± 54 mg and 55 ± 17 mg after saline and MDL 73404 respectively. These findings correlate well with the distribution of [^{14}C] labelled MDL 73404, which was rapidly distributed from a sc injection site, heart concentrations of radioactivity were maximum 1 h after injection, and remained essentially constant up to 3 h. Analysis of radioactivity in heart homogenates, by HPLC with radioactivity detection, showed that the major compound present at all time intervals studied was MDL 73404. Results showed marked cardioselectivity of radioactivity after MDL 73404, for example at 1 h after sc administration the heart tissue to blood ratio was 39.5 ± 10.5, a phenomenon which is also found with other quaternary substitued compounds (Srivastava et al., 1990). It is possible that cardioselective quaternary amine compounds may be recognized as cations by the sodium-calcium exchange system and are thus transported into the myocyte. In conclusion, MDL 73404 would seem to maximise the myocardial salvage achieved by reperfusion of the ischaemic myocardium.

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177P A RABBIT MODEL OF HEART FAILURE INDUCED BY CHRONIC RAPID VENTRICULAR PACING, AND THE ASSOCIATED ELECTROPHYSIOLOGICAL EFFECTS

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A model of heart failure produced by chronic rapid ventricular pacing has been well documented in dogs (Armstrong et al, 1986). We have attempted to adapt this methodology for use in rabbits. Rabbits implanted with pacing leads can be used to measure local repolorisation and refractoriness of the heart in vivo (Manley et al, 1989). Changes in the electrophysiological parameters during the development of heart failure may be responsible for the high incidence of sudden death seen in heart failure patients.

Twelve New Zealand white rabbits (2.8-3.2kg) were chronically implanted with bipolar pacing leads (Pacesetter Model 850F) via the right external jugular vein into the right ventricle. Subcutaneous ECG plates were also implanted. After 3-4 weeks to allow baseline electrophysiology to settle, 3 pre-treatment recordings were made and rabbits were then paced continuously at 160ms cycle length for 4 weeks. Measurements of effective refractory period (ERP) and stimulus-T (stim-T) interval of the paced evoked response were made weekly at 160ms cycle length. Stim-T has been shown to correlate closely with the action potential duration (Manley et al, 1989). Rabbit hearts were examined by echocardiography at 4 weeks pacing and clinical assessment of oedema, weight changes, dietary and water intake, and the animals' general well-being were assessed daily.

Results are means ±se mean. At four weeks the echocardiography showed a reduction in ejection fraction (63±1% to 27±2%, $p<0.001$), and an increase in left ventricular diastolic internal diameter (15.5±0.4mm to 19.7±0.4mm, $p<0.001$). In vivo electrophysiology at this time showed a decrease in ERP (100 ±2ms to 96±2ms, $p<0.05$), but no change in stim-T (118±1ms to 117±2ms, NS), compared to pre-pacing measurements. Post mortem data showed the heart weight/body weight % are increased (0.47%) compared to unpaced rabbits at the same age (0.25%, $P<0.01$) representing an increase of 88%. Rabbits had evidence of ascites, scrotal and pleural oedema, and the hearts were obviously enlarged at the time of post mortem.

Chronic rapid ventricular pacing at 160ms cycle length produces a reproducible model of heart failure and exhibits a shortening of local refractoriness which may be a causative factor of sudden death in congestive heart failure.

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178P BLOCK OF SINGLE CARDIAC SODIUM CHANNELS BY QUINIDINE

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Quinidine is an oral antiarrhythmic drug, which belongs to class Ia (Vaughan Williams, 1984) and it is effective against atrial and ventricular tachyarrhythmias. Quinidine is postulated to block Na channels in activated state (Hondeghem & Katzung, 1977; Snyders & Hondeghem, 1990). The inhibition of the Na current by quinidine is markedly frequency-dependent. The mechanisms for this action were explored in single cardiac Na channels using excised inside-out patches from adult guinea pigs.

Ventricular myocytes were obtained by collagenase dissociation (Isenberg & Klöckner, 1982). The internal and external Na concentrations were 20 and 140 mM, respectively. Electrode resistances were 6-10 MΩ. The experimental temperature was 22ºC.

Holding potentials were maintained either -110 or -160 mV. Quinidine at concentrations up to 50 μM had no effect on the amplitude of the unitary Na current, but decreased the ensemble averaged Na current when the patch was stepped from -160 mV to -40 mV by 26 %. This inhibition of the averaged current included a 67 % increase in the number of sweeps without activity. At membrane potentials more negative than -40 mV, where reopenings are likely in control, quinidine caused an increase in the number of null sweeps and, at the highest concentration of quinidine (50 μM), the mean open times (τ_o) were decreased from 1.14 ± 0.14 ms to 0.54 ± 0.04 ms (-40 mV). At lower concentrations of quinidine the τ_o did not change. Quinidine prolonged the latency to the first channel opening from 1.7 ms (control) to 3.0 ms (50 μM; -40 mV; $p < 0.05$). Similar results were obtained with lower concentrations.

These results are consistent with the notion that quinidine binds to an activated state of the Na channel, but the true open channel block occurs only at high concentrations. The increase in latency to the first opening caused by quinidine suggests that quinidine binds to an activated but closed state of the Na channel.

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179P ELECTROPHYSIOLOGICAL EFFECTS OF CRE-1087, A NEW ANTIARRHYTHMIC DRUG, IN GUINEA-PIG PAPILLARY MUSCLES

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CRE-1087, 1-(2-methoxyphenoxy)-2,3-bis (2-diethylamine-ethoxy)propane dicitrate, is a new antiarrhythmic drug which exhibits local anesthetic properties. The present experiments were undertaken to : 1) characterize the electrophysiological effects of CRE-1087, and 2) determine its effects on the onset and recovery kinetics of the frequency-dependent $\dot{V}_{max}$ block in order to place this drug in the appropriate class I subgroup (i.e., class Ia, Ib, Ic).

Experiments were performed in guinea-pig papillary muscles (less than 1 mm in diameter) perfused with Tyrode solution (34ºC). Transmembrane action potentials were conventionally recorded through glass microelectrodes filled with 3 M KCl. The maximal rate of depolarization ($\dot{V}_{max}$) of the action potential was obtained by electronic differentiation.

At concentrations higher than 10^{-7} M, CRE-1087 produced a concentration-dependent decrease in $\dot{V}_{max}$ and amplitude of the action potential but it had no effect on either the resting membrane potential or the action potential duration (APD) at both 50% and 90% of repolarization. CRE-1087, 10^{-7} M - 10^{-4} M, also prolonged the effective refractory period (ERP) and since the lengthening of the ERP was greater than that observed in the APD values, it increased the ERP/APD ratio. The onset and offset kinetics of the frequency-dependent $\dot{V}_{max}$ block were studied applying trains of pulses at different rates (0.5, 1, 2 and 3 Hz), separated from one to another by a rest period of 5 minutes. CRE-1087, 5 x 10^{-6} M, reduced by 5.0 ± 1.0 % the $\dot{V}_{max}$ of the first action potential of a train of pulses, which indicated that it exhibited a low affinity for the resting state of the Na channel. At all stimulation rates CRE-1087 increased ($p < 0.001$) the frequency-dependent block but this increase was more marked at fast (2 and 3 Hz) than at slow (0.5 and 1 Hz) frequencies of stimulation. In the presence of 5 x 10^{-6} M CRE-1087, trains of stimuli at rates between 0.5 and 3 Hz led to an exponential decline in $\dot{V}_{max}$ to a new steady-state level ($K = 0.099 \pm 0.012$ AP^{-1} and $\tau_{on} = 5.2 \pm 0.7$ s at 2 Hz). CRE-1087, 5 x 10^{-6} M, also increased the time constant of recovery of $\dot{V}_{max}$ from the frequency-dependent block (τ_{re}) to 18.3 ± 1.5 s ($p < 0.001$). The y-intercept of this slow phase of recovery which indicates the fraction of Na channels blocked by CRE-1087 was 85.4 ± 2.0 %. CRE-1087, 5 x 10^{-6} M, shifted the membrane responsiveness curve in the hyperpolarizing direction.

In conclusion, the present results demonstrated that in guinea-pig ventricular muscle fibres, CRE-1087 produced a frequency- and voltage-dependent $\dot{V}_{max}$ block. The onset and offset kinetics of the frequency-dependent $\dot{V}_{max}$ block suggested that CRE-1087 may be included in the slow subgroup (Ic) of antiarrhythmic drugs.

This work was supported by FISS and CICYT (88/0009) Grants.

180P ISCHAEMIA- AND REPERFUSION-INDUCED ARRHYTHMIAS IN ISOLATED HEARTS WITH ENHANCED α-ADRENOCEPTOR RESPONSIVENESS

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Our previous studies in anaesthetised rats have shown that the increased myocardial α-adrenoceptor responsiveness found in hypothyroid animals is not associated with an increase in the severity of ischaemia- or reperfusion-induced arrhythmias (Chess-Williams & Coker, 1989). The present study was designed to investigate whether the bradycardia present in hypothyroid animals masks any arrhythmogenic effect produced by α-adrenoceptor stimulation.

Male Wistar rats were made hypothyroid by administering 6-propyl-2-thiouracil (PTU) at a concentration of 0.4 mg ml^{-1} in their drinking water for 8 to 10 weeks. Isolated hearts from age-matched controls and PTU-pretreated animals were perfused at 70 cm H_2O by the Langendorff method with aerated Krebs-Bicarbonate solution, at 37°C. After equilibration, the coronary artery was occluded for 25 min followed by 10 min reperfusion. Hearts were either unpaced or paced at 4.3Hz throughout the experiment.

TABLE 1: Incidence and duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) during periods of ischaemia and reperfusion

	ISCHAEMIA				REPERFUSION			
	INCIDENCE(%)		DURATION(s)		INCIDENCE(%)		DURATION(s)	
	VT	VF	VT	VF	VT	VF	VT	VF
Unpaced controls	80	10	134±42	32±30	89	89	11±7	393±84
Unpaced PTU-treated	83	0	64±17	0	100	100	18±4	38±24*
Paced controls	85	85	26±5	303±81	70	100	7±2	426±65
Paced PTU-treated	60	33*	24±11	9±7*	86	86	9±3	63±39*

*($P<0.05$, Mann-Whitney U Test; n = 10-15)

The duration of reperfusion VF was reduced in spontaneous hearts from hypothyroid rats. The incidence and duration of VF was greater in paced hearts but was again reduced by PTU pretreatment.
The results indicate that even after allowing for the bradycardia observed in PTU-pretreated rats, the enhanced cardiac α-adrenoceptor responsiveness in these animals is not associated with an increase in the severity of ischaemia- or reperfusion-induced arrhythmias.

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181P THE USE OF *IN VITRO* AND *IN VIVO* RABBIT HEARTS FOR STUDIES OF THE ROLE OF ATP-DEPENDENT POTASSIUM CHANNELS DURING ISCHAEMIA

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There is speculation about the most appropriate therapy for the potentially lethal ventricular arrhythmias which arise after myocardial ischaemia. Since ischaemia-induced shortening of the cardiac action potential is pro-arrhythmic, prolongation of action potential duration (APD) by drugs which block K^+ channel subtypes is one possible approach. Of these subtypes, the delayed rectifier (whose blockers have been typified by Class III drugs such as d-sotalol or the more potent derivative, UK-66,914) and ATP-dependent K^+ channels (blockers typified by sulphonylureas, e.g. glibenclamide) are potential targets, although the possibility that Class III compounds may be an inappropriate therapy during ischaemia has been suggested by electrophysiological data (MacKenzie et al., 1990).

The case for the use of ATP-dependent K^+ channel blockers would be strengthened if they could be shown to work in some functional antiarrhythmic model, and ultimately in vivo. Here, we addressed the suitability of the rabbit for antiarrhythmic studies with K^+ channel blockers. Indirect in vitro evidence for the presence of ATP-dependent K^+ channels in the rabbit was obtained in Langendorff-perfused hearts (in Krebs solution at 37°C). The K^+ channel opener cromakalim activates ATP-dependent K^+ channels in guinea-pig ventricular muscle by a glibenclamide-sensitive mechanism (Escande et al., 1988). In isolated rabbit hearts, its isomer lemakalim (10 M) rapidly shortened extracellular APD_{50} (maximally by 75 ± 4%, n=4) and this was reversed by glibenclamide (10 M). Also, Ro 31-6930 (2 M), which is a more potent K^+ channel opener than lemakalim (Paciorek et al., 1991), produced a similar shortening of APD_{50} (by 69 ± 2%, n=4) which was reversed by glibenclamide (10 M). Coronary artery occlusion (CAO) in Langendorff-perfused hearts produced a shortening of APD_{50} and a low incidence of ventricular arrhythmias (20%, detected by ECG recording). Glibenclamide (10-100 M, n≥10) partially antagonised the reduction in APD_{50} and this effect was generally significant ($P<0.05$) at 50 and 100 M. In the virtual absence of spontaneous ischaemic rhythm disturbances, arrhythmias were induced in each preparation by a ventricular arrhythmic stimulus of variable voltage. Fibrillatory threshold voltages (V_t) were determined in the absence and presence of drugs. Glibenclamide (50 M) increased V_t from 9.5 ± 2.1V (n=12) to 21 ± 4.9V (n=11, $P<0.05$). In contrast, significant effects of the Class III agent UK-66,914 (1-20 M) under ischaemic conditions were restricted to an initial early protective effect on APD_{50} after CAO (up to 5 min), but a larger effect on V_t (44.6 ± 9V for 5 M, n=9, $P<0.05$). In the in vivo anaesthetised rabbit (Coker, 1989) CAO produced both a shortening of APD_{50} and arrhythmias (60% of controls exhibited ventricular fibrillation, VF, n=10). The ischaemia-induced shortening of APD_{50} was partially antagonised by glibenclamide (10, 20 and 30mg/kg, i.v., administered 10 min prior to CAO). The incidence of VF was reduced by glibenclamide (to 20% at 10mg/kg, n=10, insignificant) but higher concentrations (20 and 30mg/kg) resulted in mortality of the drug-treated animals due to a sudden drop in blood pressure after CAO.

The rabbit heart may possess ATP-dependent K^+ channels, which can be activated by some drugs or ischaemia. However, there are still problems associated with the use of glibenclamide as an antiarrhythmic in vivo which have to be overcome before it can be considered as a satisfactory therapeutic option for the control of ischaemic arrhythmias.

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182P CARDIOVASCULAR EFFECTS OF A_1-AND A_2-SELECTIVE AGONISTS *IN VIVO*

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Adenosine (Ado) is an endogenous nucleoside that exerts a variety of effects on cardiovascular tissues by stimulation of A_1 and A_2 adenosine receptors (Belardinelli *et al.*, 1989; Collis, 1989). These receptors elicit cardiodepression (negative inotropy, chronotropy and dromotropy) and vasorelaxation, respectively. Screening of novel Ado receptor agonists for receptor selectivity is usually performed with receptor binding assays *in vitro*, but the relevance of these classification systems to the haemodynamic effects of Ado agonists *in vivo* is unclear.

The cardiovascular effects of N^6-cyclohexyladenosine (CHA, 400-fold A_1-selective), 5'-N-ethylcarboxamidoadenosine (NECA, $A_1 \approx A_2$) and 2-phenylaminoadenosine (PAA, 5-fold A_2-selective) were compared in open-chest, fentanyl-pentobarbitone anaesthetized dogs. Graded doses of CHA (10 to 1000 $\mu g\ kg^{-1}$), NECA (0.5 to 100 $\mu g\ kg^{-1}$) or PAA (0.1 to 20 $\mu g\ kg^{-1}$) were administered intravenously and haemodynamic changes assessed 10 min following each dose. Each agent increased systemic vascular conductance index (SVCI) in a dose-dependent manner (NECA > PAA > CHA) and reduced mean arterial pressure (MAP). At doses causing similar increases in SVCI, these agonists caused (i) similar reflex increases in heart rate (HR), cardiac index (CI), cardiac inotropy (LV dP/dt_{max}) and AV conduction velocity (AV_v) and (ii) similar increases in coronary vascular conductance (CVC). These effects were antagonised by the Ado receptor antagonist, aminophylline (4 mg kg^{-1}). After cardiac autonomic blockade with atropine (0.2 mg kg^{-1}) and propranolol (1 mg kg^{-1}), CHA and PAA still increased SVCI and CVC and decreased MAP. CHA then reduced LV dP/dt_{max} but had no marked effects on CI or AV_v. PAA had no effect on LV dP/dt_{max}, HR, CI, or AV_v. As in the absence of cardiac autonomic blockade, equieffective vasodilating doses of CHA and PAA had identical effects on CVC, CI and AV_v.

Despite marked differences in receptor selectivity *in vitro*, differences between the actions of these A_1- and A_2-selective Ado receptor agonists on the cardiovascular system *in vivo* were not apparent. Difficulties therefore exist in the application of *in vitro* Ado receptor selectivity data to the prediction of haemodynamic effects of Ado agonists *in vivo*.

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183P CARDIODEPRESSANT EFFECT OF rec huTNF IN ISOLATED RAT ATRIA

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It is well established that E.Coli-induced sepsis and endotoxic shock in man (Suffredini et al,1989) and other species (Baum et al,1990) are often associated with a reduction in cardiac function. Although numerous endogenous mediators have been implicated in this phenomenon, the basic underlying mechanisms remain poorly understood. Since one of the primary mediators in endotoxin-induced shock is thought to be tumor necrosis factor α (TNF, Beutler et al,1985), a product of activated monocytes, we investigated a possible cardiodepressant effect of this polypeptide in rat isolated atrial preparations.

Male Sprague-Dawley rats weighing 250-350g were killed and the atria rapidly removed. Both atria were mounted separately under a resting tension of 0.5-1.0g (depending on size of atria) in 100ml organ baths containing Krebs' solution gassed with 95% 02 and 5% CO2 maintained at 37°C. The left atria were stimulated supramaximally at 1.5 Hz with a pulse width of 5 msec and changes in the rate of beating (right atria) and force of contraction (left atria) monitored on a Gould TA550 recorder.

Following a 60 min equilibration period, a cumulative concentration response curve (CRC) to isoprenaline (10^{-10}-10^{-6}M) was performed. Tissues were then washed for a further 60 min and a second CRC to isoprenaline performed in the absence or presence of recombinant human TNF (rec huTNF) at different concentrations ($3x10^{-12}$-$3x10^{-8}$M) given 20 min previously. To assess any differences between the 1st and 2nd isoprenaline CRCs, EC50 values were calculated and compared using a paired t test. Differences between treatments were shown by comparing dose ratios (DR) calculated for each treatment.

Isoprenaline alone gave a concentration-dependent increase in both force and rate of contraction and no tachyphylaxis was seen with the second CRC (DR = 1.16±0.19, mean ± s.e. mean). Pretreatment with rec huTNF had no effect on the isoprenaline-induced tachycardia at any concentration, nor did it modify the pre-isoprenaline basal tension. However, rec huTNF did suppress the isoprenaline-induced inotropic response causing a small but significant ($P<0.05$) rightward shift in the isoprenaline CRC. The threshold concentration was $3x10^{-11}$M (DR = 1.14 ± 0.28) and the maximum effect was seen at $3x10^{-9}$M (DR = 2.68 ± 0.55). The specificity of rec huTNF on this response was shown by administering a neutralising monoclonal antibody (Mab) against the rec huTNF polypeptide to the bath solution 15 min prior to rec huTNF. Mab pretreatment caused a concentration-dependent inhibition of the rec huTNF-induced cardiodepressant effect.

These data indicate that rec huTNF itself can affect cardiac function in vitro by depressing cardiac contractile responsiveness to isoprenaline without influencing its chronotropic effects.

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184P Org 20494, A CARDIOTONIC AGENT WITH A NOVEL MECHANISM OF ACTION

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Org 20494 (4,5-dihydro-6-(6-[2-(piperidin-1-yl)ethyloxy]-benzo[b]thien-2-yl)-5-methyl-3(2H)-pyridazinone HCl) is a novel cardiotonic agent with both cyclic AMP-dependent and cyclic AMP-independent properties. The profile of Org 20494 in vitro is described in the present study. Inotropic, chronotropic, vasorelaxant, and cyclic nucleotide phosphodiesterase (PDE) inhibitory effects of Org 20494 were evaluated as described previously (Walker et al., 1991). The effects of Org 20494 (superfused for 30 min at each concentration) on transmembrane action potential characteristics were assessed in guinea-pig electrically stimulated (1 Hz) right ventricular papillary muscles using standard microelectrode techniques.

Org 20494 increased developed tension of rabbit papillary muscles (EC_{50} 39±10 μM) and produced elongation of the relaxation phase of the twitch. These effects were concentration-related and tetrodotoxin-insensitive. In rabbit aortic rings pre-contracted with phenylephrine (0.3 μM), Org 20494 produced potent vasorelaxation (EC_{50} 1.9±0.3 μM). Cyclic AMP hydrolysis by rabbit ventricular PDE III was potently inhibited by Org 20494 (IC_{50} 0.1±0.06 μM), whilst weak effects against PDE I, II and IV were observed. Concentration-dependent negative chronotropic effects (51±6% decrease in rate at 100 μM) were observed in spontaneously beating right atria from guinea-pigs. At positively inotropic concentrations (3-100 μM), Org 20494 prolonged guinea-pig ventricular action potential duration (APD_{90} increased by 43±3% at 30 μM) with a corresponding increase in effective refractory period. The rate of depolarisation was modestly reduced at concentrations ≥ 30 μM.

Org 20494 produces in vitro positive inotropic and vasorelaxant effects which involve elevation of intracellular cyclic AMP through selective inhibition of cardiac PDE III. However, Org 20494 additionally exhibits cyclic AMP-independent properties i.e. negative chronotropism and APD prolongation. Org 20494 displays a novel profile of activity which may be useful for treating heart failure in man.

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185P EFFECTS OF Org 30029 ON THE DEVELOPMENT OF RIGOR TENSION IN TRITON-TREATED VENTRICULAR TRABECULAE OF RAT

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ORG30029 (N-hydroxy-5,6-dimethoxy-benzo[b]thiophene-2-carboximidamide hydrochloride) is a novel cardiotonic agent with positive inotropic and vascular relaxant properties. The inotropic activity has been partially attributed to selective inhibition of cyclic nucleotide phosphodiesterase isoenzymes (Shahid & Nicholson, 1990). In addition, ORG30029 (>1μM) acts directly on the myofilaments to increase Ca^{2+}-sensitivity in Triton-skinned trabeculae (Miller & Steele, 1990). A similar Ca^{2+}-sensitising action has been reported for cardiotonic agents such as pimobendan and sulmazole (Wetzel & Hauel, 1988). While the mechanism of action remains uncertain, it is generally assumed that such compounds act by increasing the affinity of troponin-C for calcium.

This study investigated the effects of ORG30029 on the development of rigor in Triton-treated rat ventricular trabeculae. Rigor develops when ATP is withdrawn from the preparation. Rigor force was independent of Ca^{2+} over the range 10^{-10}M-10^{-7}M. At higher Ca^{2+} (10^{-7}-10^{-6}M), rigor force increased steeply as a function of $[Ca^{2+}]$. In the effective absence of Ca^{2+} (10^{-10}M), addition of ORG30029 (100μM) prior to the removal of ATP caused a marked increase in the final level of rigor tension (15.5 % ±3.9, mean ± SD, n=5). However, addition or removal of the drug once the contracture had fully developed was without effect.

To explain this result by a calcium-dependent mechanism, ORG30029 would have to shift the relationship between Ca^{2+} and rigor force by at least 2 pCa units towards lower $[Ca^{2+}]$. This shift is 10 times greater than that reported for the same concentration of the drug at physiological concentrations of ATP. Alternatively, ORG30029 could affect force production by a mechanism which is primarily independent of Ca^{2+}-binding to troponin-C.

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186P THE EFFECTS OF OPIOID PEPTIDES ON CARDIOVASCULAR FUNCTION AND SYMPATHETIC NEUROTRANSMISSION IN RATS

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Endogenous opioid peptides have been proposed to play a role in cardiac arrhythmogenesis during acute myocardial ischaemia (Sitsapesan & Parratt, 1989), however, their putative mechanism of action has been little studied. One possibility is that opioid peptides may modulate sympathetic neurotransmission to the heart and blood vessels as has been shown to occur *in vitro* (Starke et al., 1985; Illes & Betterman, 1986). We have investigated the *in vivo* cardiovascular effects of the δ, μ and κ selective opioid agonists, DADLE, glyol and ICI204448 respectively in anaesthetised and in pithed rats in which their effects on sympathetic neurotransmission could also be assessed.

Rats were anaesthetised with pentobarbitone sodium or were pithed under ether anaesthesia. Mean arterial blood pressure and heart rate were measured before and following i.v. bolus injections of the drugs and/or stimulation of the thoracic spinal (C7-T2) cord. In anaesthetised rats, DADLE (δ) 10 μgkg^{-1}, glyol(μ) 0.5 μgkg^{-1} and ICI 204448 (κ) 0.1 $mgkg^{-1}$ transiently but significantly decreased heart rate from 462 ± 12 to 432 ± 14, 460 ± 12 to 448 ± 13 and 460 ± 12 to 448 ± 11 beats min^{-1}, respectively, and the mean arterial blood pressure from 142 ± 6 to 111 ± 9, 141 ± 6 to 122 ± 5, and 148 ± 7 to 121 ± 6 mmHg, respectively (n = 7 for all groups). In pithed rats, none of the opioid agonists, at these doses, altered basal cardiovascular parameters which were 273 ± 3 beats min^{-1} (heart rate) and 54 ± 1 mmHg (blood pressure). Stimulation of the thoracic spinal cord caused a stable increase in heart rate of 73 ± 2 beats min^{-1} and an increase in mean arterial blood pressure of 11.9 ± 1.9 mmHg. This change in blood pressure was not stable and declined to 4.2 ± 1.4 mmHg during continued stimulation. Higher doses of DADLE (0.5 $mgkg^{-1}$), glyol (2.5 $mgkg^{-1}$) and the same dose of ICI 2044480 (0.1 $mgkg^{-1}$) significantly reduced by 17 ± 4%, 30 ± 2% and 20 ± 10% (n = 4 or 5) respectively, the tachycardic response induced by spinal cord stimulation. This compared with a 48 ± 15% reduction with clonidine (5 μgkg^{-1}). DADLE also tended to, but did not significantly, reduce the stimulation induced increase in mean arterial blood pressure.

It is concluded that a decrease in peripheral sympathetic neurotransmission to the cardiovascular system may account at least in part, for the chronotropic effects of the opioid agonists in anaesthetised rats. Such an effect on the sympathetic nervous system is, however, unlikely to be involved in the proposed arrhythmogenic effects of the opioid peptides.

M.A. McIntosh held a SERC CASE award with Reckitt & Colman.

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187P IPAZILIDE, A NEW ANTIARRHYTHMIC DRUG: INHIBITION OF [^{3}H]-BATRACHOTOXIN BINDING TO CARDIAC SODIUM CHANNELS AND EFFECTS ON PAPILLARY MUSCLE CONTRACTILITY

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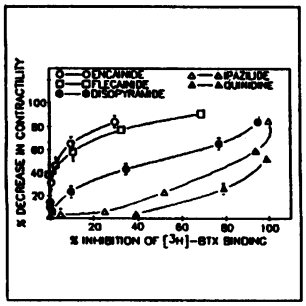
Ipazilide (N-(3-Diethylamino)propyl)-4,5 diphenyl-1H-pyrazole-1-acetamide) is a new antiarrhythmic drug now in clinical trials. Its electrophysiological action includes a primary effect on increasing cardiac action potential duration and at higher concentrations an inhibition of cardiac sodium channel function (Ezrin & Volberg, 1989). In the present studies we have compared the interaction of ipazilide and standard reference antiarrhythmic agents with cardiac sodium channels with their effects on papillary muscle contractility. The affinity of ipazilide and reference agents for sodium channels was measured using the sodium channel specific toxin [^{3}H]-batrachotoxin ([^{3}H]-BTX) and standard radioligand binding techniques (Hill et al., 1988). In this assay [^{3}H]-BTX binding to membrane prepared from rat ventricular myocytes is inhibited in a concentration-dependent manner by agents interacting with specific sites on cardiac sodium channels. The effects of ipazilide and reference agents on cardiac contractility was assessed by measuring their effects on guinea pig papillary muscle using standard techniques (Silver et al., 1989). Ipazilide inhibited [^{3}H]-BTX binding in a concentration-dependent manner with an IC_{50} of 26 ± 13μM (N=3). When the IC_{50} value for ipazilide was compared with reference antiarrhythmic agents the rank order of potency was: quinidine (11μM) = flecainide (14μM) > ipazilide (26μM) = encainide (30μM) > disopyramide (42μM) > lidocaine (66μM) > procainamide (292μM). In papillary muscle ipazilide decreased contractility, but at higher concentrations than those required to inhibit [^{3}H]-BTX binding (Figure). This was in marked contrast to encainide and flecainide, where decreases in contractility were seen at significantly lower concentrations than those required to inhibit [^{3}H]-BTX binding. Taken together these data indicate that ipazilide binds to specific sites associated with cardiac sodium channels. However, unlike encainide and flecainide, minimal changes in contractility were observed with ipazilide at its effective concentration for inhibiting [^{3}H]-BTX binding.



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188P THE EFFECTS OF THE SCORPION VENOM (*BUTHUS TAMULUS*) ON NEUROMUSCULAR TRANSMISSION

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Venom of the Indian red scorpion (*Buthus tamulus*, BT) produces various cardiovascular and other abnormalities that can cause death (Choudhury & Ganguly, 1978; Murthy & Hossein, 1986). The underlying mechanisms are unknown, but other scorpion venoms contain toxins that affect ion channels and synaptic transmission. We, therefore, investigated the effects of BT on neuromuscular transmission *in vitro*.

The effects of BT were assessed on directly and indirectly stimulated chick biventer cervicis preparations. At 1 μg/ml, BT induced a 50% augmentation of the twitch responses to indirect, but not direct, stimulation; at 3 μg/ml, the augmentation was 300% after 60 min; and at 10 μg/ml, there was a transient augmentation, followed by a contracture (about 160% of control twitch height) and a reduction in twitch height. The contracture was less than 30% in directly stimulated, tubocurarine-blocked preparations, and BT (10 μg/ml) augmented twitch responses of directly stimulated preparations by less than 50%. BT (0.3-10 μg/ml) caused little change in postjunctional sensitivity as assessed by responses to exogenous acetylcholine, carbachol and KCl. BT (3-10 μg/ml) reversed partial blockade of indirect stimulation induced by tubocurarine, procaine and Mg^{2+}. On mouse hemidiaphragm preparations, BT (10 μg/ml) produced greater augmentation of twitch responses to indirect stimulation (about 150%) than to direct stimulation (about 30%). In mouse triangularis sterni preparations, BT (3 and 10 μg/ml) increased quantal content of endplate potentials, without markedly affecting the time course, amplitude or frequency of miniature endplate potentials. Perineural recording (Rowan & Harvey, 1988) of nerve action potentials revealed that BT (30 μg/ml) caused repetitive firing of nerve endings in response to single shock stimulation.

The results are consistent with BT acting mainly prejunctionally to increase the release of acetylcholine.

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189P REVERSAL BY CYSTEINE OF THE CADMIUM-INDUCED BLOCK OF NEUROMUSCULAR TRANSMISSION

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Cadmium ions (Cd^{2+}) are known to block voltage-operated calcium channels in excitable cells and to inhibit acetylcholine release from motor nerve endings (Toda, 1976; Forshaw, 1977). On long exposure of neuromuscular preparations to Cd^{2+}, the blockade of responses is only poorly reversible by excess Ca^{2+}, but can be reversed by the thiol compound cysteine (Cys). We investigated whether Cys had any direct facilitatory actions on neuromuscular transmission and whether the reversal of the Cd^{2+}-induced block by Cys was associated with increasing Ca^{2+} currents at motor nerve terminals.

Cd^{2+} (10 - 300 μM) blocked indirectly elicited twitches on mouse diaphragm preparations. Ca^{2+} (5 - 20 mM) only partially reversed the block, but Cys (1 mM) rapidly restored twitches to control levels. On chick biventer cervicis preparations, Cd^{2+} (30-100 μM) blocked responses to indirect stimulation without changing sensitivity to acetylcholine, carbachol or KCl. Cys (0.1 - 1 mM) had no effect alone, but promptly reversed Cd^{2+}-induced blockade. In intracellular recording experiments on the mouse triangularis sterni preparation, Cd^{2+} reduced endplate potential amplitude and quantal content, without affecting miniature endplate potential amplitude. Cys (1 mM) completely reversed this effect. With extracellular recording of perineural waveforms (see Braga *et al.*, 1991), Cd^{2+} (30 - 300 μM) was found to block nerve terminal Ca^{2+}-activated K^+ currents (IK,Ca) and Ca^{2+} currents. Cys (1 - 10 mM) alone had no effect on the amplitude of IK,Ca or Ca^{2+} currents, but produced a rapid and concentration-dependent reversal of the effects of Cd^{2+}. Verapamil (30 μM) markedly reduced Ca^{2+} currents but Cys (1 - 10 mM) was unable to reverse the verapamil blockade.

We conclude that Cys reverses the blockade of acetylcholine release induced by Cd^{2+} by reversing the block of nerve terminal Ca^{2+} current induced by Cd^{2+}. This is probably due to the ability of Cys to chelate Cd^{2+}.

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190P STRUCTURE/ACTIVITY RELATIONSHIPS IN THE FACILITATORY ACTIONS OF THE DENDROTOXINS

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The dendrotoxins are a group of homologous polypeptides, isolated from mamba venoms, which block neuronal potassium currents and facilitate neurotransmitter release (Harvey & Anderson, 1985). The dendrotoxins are similar in primary sequence and three dimensional structure to Kunitz-type protease inhibitors. They are 57-60 amino acids long, with three disulphide bonds between residues 5-55, 14-38 & 30-51. Two distinct regions of the dendrotoxin molecules have been proposed as functionally important for their facilitatory action: the area corresponding to the antiprotease site in the protease inhibitors, containing one conserved lysine residue (Dufton, 1985), and an area of dense positive charge due to the other three conserved lysine residues present in all facilitatory homologues. Derivatives with either a reduced 14-38 disulphide bond (in the antiprotease site) or 5-55 disulphide bond (in the lysine site) retain the overall conformation of the native dendrotoxin but with localised changes (Hollecker & Larcher, 1989). Splitting the 14-38 disulphide specifically modified the antiprotease site, whilst the lysine site remained intact. Splitting the 5-55 disulphide caused localised structural changes at both sites. Eight modified and two native dendrotoxins were compared using competitive radioligand binding studies and the chick biventer cervicis nerve-muscle preparation. In some derivatives the free sulphydryl groups were masked with iodoacetamide and were neutral in charge ("ide"), whilst others were masked with iodoacetate and were negatively charged ("ate").

Dendrotoxin I (from black mamba venom) was labelled with ^{125}I and specific binding to rat brain membranes determined (Harvey *et al.*, 1989). Native toxin I and toxin K displaced ^{125}I-toxin I from its binding site with K_i values of 8.9×10^{-11}M and 7.3×10^{-9}M respectively. The K_i values for the modified toxin I derivatives were toxin I (5-55, 30-51; ide) 5.4×10^{-10}M, toxin I (5-55, 30-51; ate) 3.5×10^{-8}M, toxin I (30-51; ate) 3.2×10^{-7}M, toxin I (14-38, 30-51; ate) 7×10^{-7}M. Toxin I (reduced; ate), in which all three disulphide bonds were split, was inactive at concentrations of up to 10^{-5}M. The K_i values for the modified toxin K derivatives were toxin K (5-55, 30-51; ide) 1.7×10^{-8}M, toxin K (5-55, 30-51; ate) 7.7×10^{-8}M and toxin K (30-51; ate) 2×10^{-7}M.

On chick biventer cervicis preparations, none of the dendrotoxins (7.14×10^{-7}M) significantly altered postjunctional sensitivity or muscle contractility. Native toxin I augmented indirectly-stimulated muscle twitches by 120±11% at 120 min and native toxin K by 119±18%. Toxin I (5-55, 30-51; ide) increased twitches by 43±6%. The remaining toxin I derivatives were inactive at 7.14×10^{-7}M. Toxin K (5-55, 30-51; ide) increased twitches by 78±21%, toxin K (5-55, 30-51; ate) by 37±11% and toxin K (30-51; ate) by 26±15%. Values are mean ± s.e.mean, n=4-8.

These results indicate that both the antiprotease and lysine sites play a functional role in the facilitatory action of the dendrotoxins, and that toxin I and toxin K respond differently to similar chemical modifications.

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191P THE ACTION OF PONALRESTAT ON NEUROFILAMENT PHOSPHORYLATION

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Experimental diabetes in the rat leads to a reduced *in vitro* phosphorylation of spinal cord neurofilaments (Pekiner & McLean, 1991). The aldose reductase inhibitor ponalrestat can prevent some aspects of neuropathy in the streptozotocin-diabetic rat. We have investigated the ability of ponalrestat to prevent the reduced neurofilament phosphorylation.

A group of female Wistar rats were rendered diabetic by a single injection of streptozotocin (50mg.kg^{-1}, i.p.). One half of the group and a control group of non-diabetic rats also received 25mg.kg^{-1}.day^{-1} ponalrestat ('Statil'; ICI 128436). Six weeks later, spinal cords were removed and neurofilament proteins (NFP) prepared according to the method of Eyer *et al.* (1989). Phosphorylation of NFP by endogenous protein kinase(s) was measured *in vitro* by incubation of NFP at 37°C for up to 30 min with 0.5μCi ^{32}P-ATP. Protein-bound radioactivity was collected on trichloroacetic acid-impregnated filter papers and measured in a gamma counter.

After 6 weeks of untreated diabetes, *in vitro* phosphorylation of NFP was significantly ($P<0.05$) reduced to 54 ± 20% (mean ± s.d.; n=7) of that in non-diabetic rats. This reduction was completely absent in diabetic rats treated with ponalrestat. The phosphorylation of NFP from ponalrestat-treated non-diabetic rats was elevated to 234 ± 65% (n=5; $P<0.01$) of that of NFP from untreated animals. Endogenous protein kinase activity was extracted from NFP of ponalrestat-treated and untreated non-diabetic rats, by incubation with 0.8M KCl for 24h at 4°C. No phosphorylation occurred in the absence of protein kinase. When protein kinase from ponalrestat-treated rats was added to NFP from untreated rats, NFP phosphorylation was not significantly different from that when protein kinase from untreated rats was added. This indicated that the main effect of ponalrestat was not on the protein kinase itself; the drug's ability to alter the endogenous phosphorylation state of the NFP or the activity of other NFP-associated enzymes is currently being studied.

The chemically unrelated aldose reductase inhibitor sorbinil, administered at a dose of 12mg.kg^{-1}.day^{-1} to produce a similar degree of aldose reductase inhibition, was without effect on NFP phosphorylation. We have therefore observed a unique effect of ponalrestat to promote the *in vitro* phosphorylation of neurofilament proteins from rat spinal cord.

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192P EXPOSURE TO POTASSIUM CHLORIDE PRODUCES A VESAMICOL-SENSITIVE INCREASE IN QUANTAL SIZE AT THE RAT NEUROMUSCULAR JUNCTION

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(-)-Vesamicol, an inhibitor of acetylcholine (ACh) uptake into synaptic vesicles, and troxypyrrolium, an inhibitor of the high-affinity, sodium-dependent, choline transport sytem have been used as tools to study the release of pre-formed and recyling synaptic vesicles at the neuromuscular junction (Marshall *et al.*, 1990; Searl *et al.*, 1990). These previous studies have relied on the use of moderate rates of nerve stimulation to induce vesicular recyling. We have now investigated the ability of the two compounds to effect pre-formed and recyling synaptic vesicles under conditions where ACh release is elevated by sustained potassium-induced nerve terminal depolarization.

Recordings of spontaneously occuring miniature end-plate currents (m.e.p.cs) were made from rat neuromuscular junctions at 32 °C using a conventional two microelectrode voltage clamp technique in cut-muscle hemidiaphragm preparations. M.e.p.cs were recorded at -55 mV for a 4 minute period prior to a 4 minute exposure of the tissue to 25 mM KCl. Following the 4 minute exposure to KCl the preparation was washed and m.e.p.cs were recorded for a further 4 minutes. The post-KCl m.e.p.cs were analyzed over a 40 second period starting from the point at which the m.e.p.c frequency was reduced to the level where individual events were discernable (30 - 60 seconds following initiation of washout).

Unexpectedly, following exposure to KCl mean m.e.p.c amplitude was increased from 2.98 ± 0.36 nA to 3.81 ± 0.25 nA (n=5, $P < 0.05$, paired Student's *t* test). This was not accompanied by any change in the distribution of m.e.p.c. amplitudes (control coefficient of variation (CV), 19.0 ± 1.6 %; post-KCl, 19.6 ± 0.8 % (n=5)). The KCl-induced increase in m.e.p.c. amplitude was not seen in the presence of 1 μM (-)-vesamicol (pre-KCl, 2.69 ± 0.36 nA; post-KCl, 2.59 ± 0.21 nA (n=5)) or 20 μM troxypyrrolium (pre-KCl, 2.58 ± 0.37 nA; post-KCl, 2.78 ± 0.35 (n=5)).

We have previously shown, in the rat (Marshall *et al.*, 1990), that 1 μM (-)-vesamicol blocks synaptic vesicle recycling with no apparent effects on either pre-formed (depot) synaptic vesicles or on post-junctional sensitivity to acetylcholine. Therefore, the ability of 1 μM (-)-vesamicol to inhibit the KCl-induced increase in m.e.p.c. amplitude suggests that the KCl-induced increase is a prejunctional phenomenon to increase acetylcholine packaging into synaptic vesicles. The similar CV for the m.e.p.c. amplitudes before and after exposure to KCl suggests that this increase in packaging occurs in all synaptic vesicles, both recycling and pre-formed. The sensitivity of the KCl effect to troxypyrrolium suggests that the increase in quantal size was due to the packaging of newly synthesised transmitter. The ability of (-)-vesamicol to inhibit the effects of KCl is evidence that, in addition to its established effects on recycling synaptic vesicles (Marshall *et al.*, 1990; Searl *et al.*, 1990), the compound also inhibits the movement of acetylcholine into the pre-formed synaptic vesicles.

KP holds an MRC post-graduate studentship.

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193P ZIDOVUDINE DOES NOT IMPAIR NEUROMUSCULAR TRANSMISSION IN THE RAT PHRENIC NERVE DIAPHRAGM PREPARATION

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Zidovudine (3'azido-3'deoxythymidine) is an antiretroviral drug effective in reducing the incidence of opportunistic infections and neoplasms in patients with AIDS, resulting in improved survival rates (Fischl *et al.*, 1987). During treatment with standard doses (zidovudine 1 g per day) approximately 10% of patients develop proximal muscle weakness, fatigue, aching and stiffness. Using the rat phrenic nerve diaphragm preparation the effect of zidovudine on the twitch height of the striated muscle of the diaphragm in response to electrical stimulation of the phrenic nerve was studied in the presence and absence of the competitive neuromuscular blocking agent tubocurarine.

The rat phrenic nerve diaphragm preparation was mounted in a tissue bath containing 50 ml of Krebs solution, well aerated with oxygen (95%) and carbon dioxide (5%) and maintained at a temperature of 35.5°C and a pH of 7.5. The phrenic nerve was stimulated by a 2 V shock, 0.5 msec duration at a frequency of 0.1 Hz. Zidovudine at concentrations of 5, 10 and 20 μmol l^{-1} did not alter the twitch height. Table 1 shows the effect of zidovudine (10 μmol l^{-1}) when added to preparations pre treated with varying concentrations of tubocurarine (1.5, 2.0 and 3.0 μg ml^{-1}).

Table 1 Effect of zidovudine on twitch height in the presence of tubocurarine

	% of control twitch height		P Value
tubocurarine μg ml^{-1}	- zidovudine	+ zidovudine (10 μmol l^{-1})	
1.5	84.9 ± 5.0	86.0 ± 7.2	P > 0.05
2.0	73.9 ± 5.2	74.0 ± 9.7	P > 0.05
3.0	56.9 ± 9.1	56.0 ± 9.0	P > 0.05

Results are mean ± s.d., n = 6, Students t-test

These results demonstrate that zidovudine (10 μmol l^{-1}) does not influence the rat phrenic nerve diaphragm preparation even in the presence of tubocurarine. This suggests that interference with neuromuscular transmission is unlikely to contribute to zidovudine induced muscle weakness and fatigue which has been attributed solely to a zidovudine myopathy which is distinct from HIV-1 associated myopathy (Till & McDonnell, 1990).

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194P THE EFFECTS OF HAEM SYNTHESIS BLOCKING AGENTS ON THE RESPONSES OF SOME RAT ISOLATED NERVE/MUSCLE PREPARATIONS

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The porphyrias are a group of disorders of haem metabolism. The symptoms of the acute porphyrias suggest a central and peripheral neuropathy. One hypothesis implicates a deficit in essential haemoproteins in the aetiology of these neurological symptoms (Moore et al., 1987). A reduction in haem availability results in a shrinkage of a regulatory haem pool causing a rise in the activity of the rate limiting enzyme, 5-amino-laevulinic acid synthase (ALAS) and subsequent elevated ALA.

Several compounds disrupt the equilibrium of haem synthesis. Succinyl acetone inhibits ALA dehydratase (Sassa and Kappas, 1983). 3,5-Diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-ethylpyridine (4-ethyl DDC) mechanistically destroys the haemoprotein cytochrome P-450, and the product, an N-alkylated protoporphyrin IX, inhibits ferrochelatase (Ortiz de Montellano, 1981). Phenobarbitone induces cytochrome P-450 synthesis and inhibits uroporphyrinogen decarboxylase (De Verneuil, 1983). The present experiments examine whether such drugs interfere with peripheral nerve-muscle function using a range of preparations to evaluate cholinergic, adrenergic and NANC transmission.

Male Wistar rats received either short or long term porphyrinogenic drug treatment. Short term treatment consisted of twice daily administration of succinyl acetone (40mg/kg) for three days, while long term treatment comprised SA (20mg/kg) administration for 30 days followed by 4-ethyl DDC (25mg/kg) 10 days, 4 days phenobarbitone (80mg/kg) and finally 4-ethyl DDC (300mg/kg); SA administration was continuous throughout this treatment. All drugs were administered i.p.

Urinary ALA levels were elevated by both combinations throughout the treatment. There was no observed autonomic neuropathy as measured by the responses of muscles to field stimulation of their intrinsic nerves, following either treatment. This was the case for the anococcygeus muscle motor response, a noradrenergically-mediated event and for the inhibitory action of this muscle, a response mediated by an NANC transmission. The biphasic response of the vas deferens, a purinergically and noradrenergically-mediated phenomenon was normal as was the pressor response of stimulated perfused tail artery, a response mediated by co-transmitters NA and possibly ATP. The cholinergically-mediated response of the diaphragm to phrenic nerve stimulation did not differ significantly from control tissues. The results indicated that although both treatment regimes disrupt haem synthesis to the extent of reducing the uncommitted haem pool with a subsequent derepression of ALAS activity, essential haemoproteins were not compromised to a level where a neuropathy ensues.

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195P DIFFERENTIAL EFFECTS OF HAEM SYNTHESIS BLOCKING AGENTS ON ERYTHROCYTIC, HEPATIC AND NEURAL HAEMOPROTEIN CONTENT

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It has been proposed that the underlying cause of acute porphyric neuropathy is a deficit in essential haemoproteins, particularly the respiratory cytochromes (Yeung Laiwah, 1987). Several compounds disrupt haem biosynthesis. Succinyl acetone (SA), inhibits 5-aminolaevulinic acid dehydratase (ALAD) (Lindblad, 1977). 3,5-Diethoxycarbonyl-1,4-dihydro-2,6-dimethyl-4-ethylpyridine (4-ethyl DDC) destroys cytochrome P-450 by a mechanism-based inactivation producing an N-alkylated protoporphyrin IX which inhibits ferrochelatase (Marks, 1987). Phenobarbitone induces cytochrome P-450 and inhibits uroporphyrinogen decarboxylase (De Verneuil, 1983). The present experiments examine whether these drugs cause a fall in tissue haemoproteins.

The haemoprotein content of erythrocytes (haemoglobin, catalase), liver (catalase, respiratory cytochromes b, c, c^1 and $a+a_3$) and brain (respiratory cytochromes) were measured following administration of porphyrinogenic agents. Male Wistar rats received four treatment combinations. Treatment 1: 30 days i.p. administration of SA (20mg/kg). Treatment 2: 30 days SA (20mg/kg), followed by 10 days 4-ethyl DDC (25mg/kg), 4 days phenobarbitone (80mg/kg) and finally 300mg/kg 4-ethyl DDC; SA was continued throughout the treatment (i.p. administrations). Treatment 3: 14 days 4-ethyl DDC (100mg/kg) i.p.. Treatment 4: 14 days N-methyl protoporphyrin IX (1μM, i.v.).

SA alone had no adverse effect on any haemoproteins measured. Treatment 2 resulted in a significant drop in hepatic catalase and respiratory cytochromes b, c, c^1 and $a+a_3$ content. Treatment 3 produced a significant depression of hepatic catalase activity and cytochrome b,c and a+a3 content. Treatment 4 caused a significant decrease in hepatic catalase and cytochrome oxidase. The content of all other haemoproteins was uncompromised. Brain haemoproteins were unaffected by all combinations when compared to controls. In hepatic tissue only SA failed to reduce haemoprotein content. This is probably due to the low ALAD activity compared to that of the next enzyme in the pathway, porphobilinogen deaminase and although reduced enough product is produced to maintain adequate haem production. The differential action of porphyrinogenic agents on hepatic and erythrocytic haemoproteins is most likely due to their different half-lives: hepatic catalase 2 days; respiratory cytochromes 6 days; erythrocytes 60 days. There was no evidence of a condition corresponding to acute porphyria in man. This may be linked to the failure to reduce haemoproteins in the nervous tissue and this in turn to a difficulty for some of these drugs to cross the blood brain barrier.

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196P EFFECTS OF A BOMBESIN ANALOGUE ON NEUROMEDIN B- AND BOMBESIN-INDUCED CALCIUM FLUX IN THE SMALL CELL LUNG CARCINOMA LINE NCI-H345

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Neuromedin B (NMB) is a peptide structurally related to bombesin (BN) and gastrin-releasing peptide (GRP) (Minamino *et al.*, 1988). GRP has been implicated as a growth factor in small cell lung carcinoma (SCLC). In this study, we examined the effect of NMB and BN on Ca^{2+} flux in the SCLC line NCI-H345. NCI-H345 cells, incubated with fura-2-AM, were placed in a fluorimeter where the change in Ca^{2+} was measured in the presence of NMB or BN. Both peptides elevated Ca^{2+} in a concentration-dependent manner. As with BN (Heikkila *et al.*, 1987), acute desensitisation was associated with NMB.

Table 1 Effects of a BN analogue on NMB- and BN-induced calcium flux

Treatment	EC_{50} (nM)	E_{max} (%Δ[Ca^{2+}])
NMB	6.0 ± 1.2	159 ± 10
NMB + DPDM-BN EA (100 nM)	2.3 ± 0.34	116 ± 7.8*
BN	4.8 ± 1.1	160 ± 5.9
BN + DPDM-BN EA (100 nM)	32 ± 9.0†	157 ± 14

Results are means ± s.e. mean (n=3-6). *P < 0.05 vs. NMB, †P < 0.05 vs. BN (Student t-test).
EC_{50} and E_{max} were calculated by an iterative curve fit program (Kaleidagraph, Synergy Software).

Effects of the BN antagonist, [D-Phe^6, Des-Met^{14}]-BN (6-14) ethylamide (DPDM-BN EA), on NMB- and BN-induced Ca^{2+} transients are shown in table 1. DPDM-BN EA (100 nM), inactive alone, competitively antagonised BN-induced Ca^{2+} flux, yet its interaction with NMB was different. This is the first reported evidence of a concentration-dependent effect by NMB, as well as antagonism of a NMB-induced effect by a BN analogue, on SCLC. Furthermore, the pharmacological profiles imply the existence of receptor subtypes for the BN family of peptides on the SCLC cell line NCI-H345.

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197P ON THE PRECLINICAL PHARMACOLOGY OF L-685,393 AND MK-507, TWO TOPICALLY EFFECTIVE OCULAR HYPOTENSIVE CARBONIC ANHYDRASE INHIBITORS

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Systemically administered carbonic anhydrase (CA) inhibitors lower the elevated intraocular pressure (IOP) of glaucoma patients. However, patient compliance is poor due to unwanted side effects stemming from the extraocular inhibition of CA. In spite of intensive effort in the past, only recently have clinically effective, topical CA inhibitors become available. The preclinical pharmacology of MK-927 (Sugrue et al., 1990a) and MK-507 (L-671,152; Sugrue et al., 1990b) was such that both agents were evaluated in patients with glaucoma or ocular hypertension and were found to be active (Bron et al., 1989; Lippa et al., 1991).

L-685,393 ([-]-5,6-dihydro-4-ethylamino-6-[n-propyl]-4H-thieno[2,3-b]-thiopyran-2-sulfonamide-7,7-dioxide HCl) is a water-soluble CA inhibitor with a chemical structure similar to that of MK-507. The IC_{50} values of L-685,393 and MK-507 against CAII isolated from human red blood cells were 0.26 nM and 0.20 nM, respectively. The acute instillation (one drop, 50 µl) of 0.5% and 1% solutions of L-685,393 maximally decreased the IOP of ocular hypertensive, ketamine-tranquillized cynomolgus monkeys by 8.3 mm Hg and 10.3 mm Hg, respectively. The peak reductions in the IOP of these animals after the instillation of 0.5% and 1% solutions of MK-507 were 8.6 mm Hg and 11.8 mm Hg, respectively. Thus, both compounds are of comparable potency in this paradigm. However, the compounds differed in their duration of action. The IOP of ocular hypertensive monkeys was decreased by 3.1 mm Hg at 16 hr after the instillation of 2% MK-507. In contrast, the decline in IOP (11.2 mm Hg) was significantly greater at this time point following the instillation of 2% L-685,393. At all time points out to, and including, 22 hr post-dosing, the decrease in IOP was greater after 2% L-685,393 than after 2% MK-507. The instillation of 2% L-685,393 maximally lowered the IOP of ocular normotensive, albino rabbits by 4.0 mm Hg at 2 hr. A 1-hr pretreatment with 2% L-685,393 significantly decreased aqueous humor inflow in albino rabbits by 40%. In contrast, a similar treatment was essentially devoid of effect (+ 10%) on aqueous humor outflow.

In summary, L-685,393, like MK-507, is a topical CA inhibitor possessing ocular hypotensive activity by virtue of a reduction in aqueous humor inflow. A key difference in the pharmacological profiles of the two compounds is the longer duration of action of L-685,393.

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198P THE EFFECT OF TIME AND DIFFUSION CELL RECEPTOR FLUID ON ESTERASE ACTIVITY IN HAIRLESS MOUSE SKIN *IN VITRO*

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The bioavailability of many drug preparations applied to the skin are often tested in vitro using diffusion cells. There are various designs used (Skelly et al 1987) which all consist of a donor compartment and receptor compartment separated by a membrane such as animal or human skin. The receptor fluid most commonly used is phosphate buffered saline [PBS] which may be either stirred in a static cell or pumped through in a flow cell. The release profile of a drug which is of fundamental importance, will be affected in vitro by the lipophilicity of the drug, its diffusion characteristics and partitioning of the drug between the drug preparation and the skin barrier. These processes would be affected by alteration of the drugs characteristics by skin metabolism. Most studies in vitro assume that the skin is a passive barrier and ignore any potential contribution from enzyme processes.
In this study mouse skin was incubated in contact with either PBS, or tissue culture medium (GIBCO BGJ=B) at 32°C for six hours. Some samples were aerated hourly with 95% O_2/5% CO_2. Subcellular fractions were prepared from the skin samples and p-nitrophenyacetate (PNPA) esterase activity estimated as described (Keysell & Panchal 1990).

Table 1. PNPA esterase activity in hairless mouse skin (n moles/min), standard deviation

Skin conditions	O_2CO_2	Specific activity (per mg protein) Cell cytosol	microsomes	Total activity (per g tissue) Cell cytosol	microsomes
Fresh	NO	190 ± 9	386 ± 23	4940 ± 360	1864 ± 149
in PBS	NO	156 ± 9	324 ± 26	2717 ± 190	1328 ± 106
in PBS	YES	152 ± 8	331 ± 27	2736 ± 195	1374 ± 112
in culture media	NO	154 ± 9	330 ± 25	2816 ± 183	1336 ± 113
in culture media	YES	162 ± 10	385 ± 31	2915 ± 210	1597 ± 118

The results, Table 1, indicate that there were no significant differences between the 2 media used in the receptor compartment although total activity in the aerated microsomal fraction was significantly higher than the non aerated sample ($p<0.05$). Differences between in vivo and in vitro permeation of a salicylic ester have been attributed to differences in the rate of hydrolysis and subsequent removal of the drug in the skin (Potts et al 1989). In this study, with both receptor fluids, esterase activity after six hours, was still more than 50% of the original activity found in freshly excised skin and could therefore influence the in vitro permeation kinetics of drugs susceptible to hydrolysis.

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199P EXPRESSION OF DIFFERENTIATED HEPATIC FUNCTION IN SV40-IMMORTALISED RAT LIVER CELLS

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Differentiated cell lines can be obtained by transfecting primary cells with DNA containing viral oncogenes. Immortalised liver cell lines obtained in this way are a potential alternative to primary rat hepatocytes for studying xenobiotic metabolism and toxicity in vitro, if they retain stable expression of parenchymal functions.

As indicators of differentiated function, the activities of γ-glutamyltransferase (γ-GT), the π isoenzyme of glutathione-S-transferase (π-GST) and the presence of the cystathionine pathway for glutathione (GSH) synthesis were compared in freshly isolated Sprague-Dawley rat hepatocytes, the rat hepatoma cell line HTC and the SV40-immortalised rat liver cell line SV40RH1 at passage 5 and 13. SV40RH1 cells were grown on hydrated collagen gels in a hormonally-defined medium based on Ham's F12/Williams' E.

The results, Table 1, indicate that the immortalised line retained the ability to synthesise GSH from cysteine at both passage 5 and 13, but lost the ability to synthesise GSH from methionine at passage 13. High levels of GSH synthesis from cysteine were also observed in two other SV40-immortalised lines (data not shown). GST activities were measured in the presence of 1mM GSH using either the general substrate 50μM 1-chloro-2,4-dinitrobenzene (CDNB) or 0.2mM ethacrynic acid (EA) as substrate to detect the π isoenzymic form, and the results are expressed as the ratio of the two activities. Although this ratio dropped between passage 5 and 13, it is important to note that levels of another marker studied, γ-GT, remained undetectable in fresh hepatocytes and SV40RH1 at both passage 5 and 13, whereas HTC contained 5.25 ± 0.72 nmol/min/mg (n=3).

Table 1 Enzyme activities in hepatocytes, HTC cells and the immortalised line SV40RH1

Enzyme	SV40RH1 pass 5	SV40RH1 pass 13	HTC	Hepatocytes
Ratio CDNB/EA activities	6.54±1.28(4)	0.14±0.04(12)	0.22±0.04(5)	9.32±1.71(4)
Rate of GSH synthesis: L-cys (nmol/mg protein/min)	0.35±0.06(3)	0.57±0.1 (3)	0.13±0.01(3)	0.22±0.01(5)
: L-met	0.21±0.04(3)	0 (3)	0.01 (3)	0.18±0.05(5)

Values are expressed as mean ±SD, with the number of experiments in parentheses.

These results indicate that immortalised rat liver cells retain aspects of the phenotype of fresh hepatocytes even after 13 passages in culture

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200P THE TOXICITY OF CYCLOPHOSPHAMIDE IN HepG2 HEPATOMA CELLS AND CULTURED RAT HEPATOCYTES

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The cytotoxicity of the anticancer agent cyclophosphamide, which is clinically hepatotoxic (Goldberg and Lidsky, 1985), was investigated in cultures of rat hepatocytes and the human hepatoma HepG2 cell line.

HepG2 cells were grown in Williams' E medium. Hepatocytes were isolated from male Sprague Dawley rats and cultured in Modified Earles Medium. The acute toxicity of cyclophosphamide was investigated by exposing HepG2 cells or cultured rat hepatocytes to cyclophosphamide for 16h. Viability was determined using Trypan blue or lactate dehydrogenase leakage. Rats and HepG2 cells were treated, *in vivo* or in culture respectively, with the cytochrome P450 inducers phenobarbitone (PB), benz(a)anthracene (BA), rifampacin (R) or pregnenolone-16α-carbonitrile (PCN).

Cyclophosphamide toxicity was greatest in hepatocytes from rats induced with PB or PCN, consistent with metabolic activation by P450IIB (Clarke and Waxman, 1989) and possibly by P450IIIA. Cyclophosphamide was less toxic to HepG2 cells than to rat hepatocytes, perhaps due to a relative absence of the activating form of P450 from HepG2 cells. In HepG2 cells R caused a small but significant increase in toxicity, indicating possible activation by induced P450IIIA. The inhibitors of P450, metyrapone and ketoconazole, each increased the toxicity of cyclophosphamide in HepG2 cells, suggesting that in these cells P450 also detoxified the drug. In HepG2 cells cyclophosphamide depleted reduced glutathione (GSH), but this *succeeded* cell death, while the drug's toxicity was not affected by depletion or repletion of GSH using buthionine sulfoximine and L-cysteine respectively. Although the toxicity of cyclophosphamide is considered to be due to the breakdown-product of cyclophosphamide metabolism, acrolein, which is detoxified by GSH (Crook *et al*, 1986), in HepG2 cells the mechanism of cyclophosphamide toxicity appears not to involve GSH.

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201P THE INVOLVEMENT OF NITRIC OXIDE IN NEURONALLY MEDIATED RELAXATION IN THE RABBIT DISTAL COLON

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Nitric oxide (NO) has been proposed as the inhibitory neuro-transmitter from non-adrenergic, non-cholinergic (NANC) nerves in the rat anococcygeus (Gillespie et al., 1989) and bovine retractor penis muscle (Gillespie & Sheng, 1989), and in the canine lower oesophageal sphincter (De Man et al., 1991) and ileo-colonic junction (Boeckxstaens et al., 1991). The possibility of NO having an inhibitory role in gastrointestinal smooth muscles has now been investigated.

Circular muscle strips of the rabbit distal colon (RDC) were prepared for isometric tension recording (Pelckmans et al., 1989) in a jacketed (25ml) organ bath at 37±1°C in the presence of phentolamine and propranolol (each $3x10^{-6}$M). Stimulation was achieved using Ag/AgCl ring electrodes (0.4cm o.d.) by passing square wave pulses at supramaximal voltage (40V 0.1-0.5ms at 0.5-16Hz). Following the development of carbachol-induced tone ($3x10^{-5}$M), electrical stimulation produced a frequency dependent (4Hz optimum), TTX (10^{-6}M)-sensitive relaxation and rebound contraction. NO (as a solution of the gas > 10^{-6}M, prepared as described by Gillespie & Sheng, 1988) relaxed the RDC. Nω-Nitro-L-Arginine methyl ester (L-NAME $5x10^{-4}$M) significantly reduced ($P<0.05$) but did not abolish the NANC mediated relaxation (16 pulses, 0.5-16Hz, 0.1-0.5ms, 40V). The L-NAME-mediated inhibition was reversed by L-Arginine (>$2x10^{-4}$M)-a precursor of NO synthesis. The bee venom, apamin ($5x10^{-7}$M) also reduced the amplitude of the neuronal response.

Oxyhaemoglobin (3-$5x10^{-5}$M) which binds NO, reduced the size of the NANC nerve-mediated relaxations (16 pulses, 0.5-8Hz, 0.2-0.3ms). Responses to exogenous NO ($2x10^{-5}$M) were abolished in the presence of oxyhaemoglobin ($3x10^{-5}$M). The results suggest that NO contributes to NANC mediated relaxation in the RDC.

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202P NORADRENALINE AND ENDOTHELIN COUPLING TO IP FORMATION IN CULTURED AORTIC CELLS FROM SPONTANEOUSLY HYPERTENSIVE RATS

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Noradrenaline (NA) and endothelin responses are mediated in part by the phosphatidylinositol (PI) cycle. In vascular smooth muscle activation of this cycle by these agonists is mediated by GTP-binding proteins (G-proteins) (LaBelle and Murray, 1990; Reynolds et al, 1989). The development of hypertension may be related to changes in this signal transduction pathway (Sharma and Bhalla, 1988). We have measured inositol phosphate (IP) formation with NA and endothelin in smooth muscle cells cultured from the aortae of 6 and 14 week spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats and examined the effect of pertussis toxin which inhibits certain subtypes of G-proteins. Cultured cells (passages 4-10) were pre-incubated with [^{3}H]-myoinositol for 18 hours and incubated with either NA or endothelin for a further 30 minutes. In experiments with pertussis toxin (0.1 ug/ml) cells were pre-incubated for 18 hours. Total IP formation was measured following purification on Dowex columns. All results are mean ± s.e.m. ANOVA was used to compare WKY and SHR responses (n=6-9).

Both NA (10^{-7}-10^{-4}M) and endothelin (10^{-12}-10^{-6}M caused a concentration-dependent increase in IP formation. In cultured cells from 6 week rats SHR and WKY responses were similar with NA and endothelin. In cells from 14 week SHR the IP formation with NA was significantly increased compared to WKY (10^{-4}M : WKY 204±33, SHR 271±26% of basal). However, the endothelin stimulated IP formation in the cells from 14 week rats was decreased in SHR compared to WKY (10^{-6}M : WKY 2450±504, SHR 927±137% of basal). Basal IP formation in SHR cultured cells was greater than WKY at both time points (at 6 weeks - WKY=312±64, SHR=671±122; at 14 weeks - WKY=342±88, SHR=870±148 cpm/10^5 cells). Pre-incubation with pertussis toxin had no effect on basal IP formation or on NA stimulated IP formation in cultured cells from 14 week SHR and WKY. Endothelin stimulated IP formation was significantly reduced by 45% in both 14 week SHR and WKY cells after pre-incubation with pertussis toxin. In conclusion both NA and endothelin stimulated IP formation is changed in cultured aortic smooth muscle cells from 14 week old SHR although this change is in opposite directions. NA and endothelin coupling to the PI cycle is regulated in part by different G-proteins in this system and this independent regulation may explain the agonist specific responses observed in these hypertensive cultured smooth muscle cells.

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203P A COMPARISON OF THE EFFECTS OF ISOPRENALINE, ACETYLCHOLINE AND ISOBUTYLMETHYLXANTHINE ON NORADRENALINE-INDUCED TONE AND PI HYDROLYSIS IN RAT AORTA *IN VITRO*

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Tone in vascular smooth muscle can be inhibited by drugs that activate adenylate cyclase or guanylate cyclase (Lincoln & Fisher-Simpson, 1983; Rapoport, 1986). This study compared the effects of isoprenaline (ISO) with those of acetylcholine (ACh) and isobutylmethylxanthine (IBMX) on noradrenaline (NA)-induced tone and phosphoinositide (PI) hydrolysis in rat aortic rings (3-4 mm), which were suspended under 2 g resting tension in Krebs buffer at 37°C and gassed with 95% O_2/5% CO_2. NA-induced PI hydrolysis was measured in rings, labelled with (myo-^{3}H)-inositol (8 μCi ml^{-1}) and incubated in Krebs buffer containing lithium chloride (10 mM) and NA for 1 h at 37°C. After incubation the aortic rings were homogenised and inositol phosphates were extracted, separated chromatographically on Dowex formate resin and measured by liquid scintillation counting. Concentration-response curves for the contractile response to NA (nM-30 μM) and for the PI response to NA (10 nM-mM) were obtained. NA (6.2 μM, EC_{75}) increased PI hydrolysis from the basal level, 79.3 ± 4.4 (dpm mg^{-1} tissue, mean ± s.e. mean, n=8) to 648.6 ± 32.6 (n=8, P<0.001). This response was reduced by ISO (μM) to 478.5 ± 17.8 (n=6, 0.01>P>0.001) and this effect was antagonised by propranolol (PROP, 5 μM), which restored the NA-induced PI response to 641.3 ± 52.2 (n=7). ISO (μM) abolished NA-induced tone in aortic rings. ACh (10 μM) reduced the NA-induced PI response from 502.2 ± 30.9 (n=7), 0.01<P<0.001) in intact aortic rings in which the basal PI response was 67.4 ± 7.8 (n=6). This concentration of ACh inhibited NA-induced tone by 80% when the endothelium was present but had no such inhibitory effect when the endothelium was removed by rubbing. In such tissues, ACh (10 μM) did not inhibit the NA-induced PI response (526.6 ± 46.9, n=7, N.S.). IBMX (10 μM) also inhibited the NA-induced tone by approximately 80% and reduced the NA-induced PI response from 437.2 ± 38.7 (n=7) to 330.9 ± 27.2 (n=7, 0.05>P>0.01) in experiments where the unstimulated basal PI response was 80.6 ± 6.9 (n=9). These results indicate that concentrations of ISO, ACh and IBMX, which abolish or greatly reduce NA-induced tone in the rat aorta, have less marked inhibitory effects on the NA-induced PI response. This disparity occurs whether the effects of the vasorelaxant are mediated by cyclic adenosine monophosphate (ISO), cyclic guanosine monophosphate (ACh) or both nucleotides (IBMX). Since the phosphodiesterase inhibitor, IBMX relaxed NA-induced tone and inhibited the NA-induced PI response, it appears that cyclic nucleotide synthesis continues during NA-induced contractions.

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204P INVESTIGATION OF THE EFFECT OF PROTEIN KINASE C ACTIVATION ON ANF-STIMULATED CGMP PRODUCTION IN LLCPK$_1$ CELLS

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It has been reported in the literature that activators of protein kinase C (PKC) negatively regulate the atrial natriuretic factor (ANF)-stimulated guanylate cyclase system (Jaiswal & Sharma, 1988; Nambi et al., 1987). In addition, the potent effects of PKC on cell proliferation, coupled with a proposed antimitogenic action of agents which stimulate cGMP production (Garg & Hassid, 1989) may further suggest an interaction between ANF and PKC at membrane level. In this study we have established the presence of PKC activity in LLCPK$_1$ cells (an established kidney epithelial cell line) and we have assessed the ability of this enzyme to modulate ANF-stimulated cGMP production.

Cytosolic and membrane fractions of confluent LLCPK$_1$ cells were prepared after cell harvesting by scraping of monolayers, pelleting, cell disruption by Dounce homogenization and centrifugation at 15,000 x g for 15 min at 4°C. The supernatant was designated the cytosolic fraction and the pellet was rehomogenized, treated with 0.1% Triton X-100 and recentrifuged as above. The resulting supernatant was designated the Triton X-100 extract of the membrane fraction. Following ion exchange chromatography of both fractions on DE-52 cellulose, PKC activity was measured as the incorporation of phosphate from τ-[^{32}P]ATP into the exogenous substrate histone III-S in the presence of necessary cofactors (Ca^{++}, phosphatidylserine, diolein). Specific binding of 15 nM [^{3}H]-PDBu (phorbol 12,13-dibutyrate) was measured in intact cells and was displaceable by 30 μM PDBu. Cellular cGMP accumulation under basal and ANF-stimulated conditions was quantified by radioimmunoassay, following extraction of the nucleotide from cells with 0.1 M HCl. Results are presented as mean values +/- s.e.m. of n observations.

Stimulated PKC activities in cytosolic and membrane fractions isolated from LLCPK$_1$ cells were 8.0 ± 0.8 and 3.7 ± 0.9 pmol phosphate transferred/min/mg protein, respectively (n=3). Basal cellular cGMP accumulation was 3.86 ± 0.76 pmol/5 min/10^6 cells and $rANF_{99-126}$ (1 μM) increased this value by 3.00 ± 0.47 fold, (n=8). Pretreatment of cells with 1 μM PDBu for 1 hr did not affect ANF-stimulated cGMP production but the relative distribution of PKC activity between cytosol and membrane was altered by this pretreatement (58% of activity now being present in the membrane). Furthermore, the specific binding of [^{3}H]-PDBu to intact cells was reduced by 51% (n=2) following the pretreatment.

We conclude from this study that the lack of ability of the PKC activator PDBu to negatively regulate ANF-stimulated cGMP accumulation may be due to redistribution and/or downregulation of the enzyme in LLCPK$_1$ cells.

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205P EFFECTS OF AMYLIN AND CGRP ON THE ADENYLATE CYCLASE/CYCLIC AMP SYSTEM IN RAT HEPATOCYTES

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Amylin (diabetes associated peptide; islet amyloid polypeptide) is a 37 amino acid neuropeptide-like molecule which is co-secreted with insulin from the beta cells of the pancreas. It is the major component of islet amyloid deposits found in the pancreases of non-insulin-dependent (type II) diabetics (Leighton & Cooper, 1990), can cause insulin resitance, *in vivo*, and may have a role in the induction of insulin resistance in humans (Molina, *et al*, 1990). The mechanism of action of amylin is not fully understood. Whilst it has been proposed that amylin exerts its effects on the liver by binding to putative calcitonin gene related peptide I (CGRP) receptors (Yamaguchi *et al*, 1988) and increasing intracellular cyclic AMP levels, a recent report has suggested that the effects of amylin on glycogen metabolism in muscle occur via a cyclic AMP-independent mechanism (Deems *et al*, 1991).

Here, we have examined the effects of amylin and CGRP on the adenylate cyclase activity of rat hepatocyte plasma membranes and compared the effects of the competitive CGRP receptor antagonist CGRP(8-37), on the activation of adenylate cyclase by CGRP and amylin. We also examined the effects of amylin on intracellular cyclic AMP levels in hepatocytes. Hepatocytes and washed plasma membranes were prepared from male Sprague-Dawley rats and adenylate cyclase activity and intracellular cyclic AMP levels were determined as described previously (Whetton *et al*, 1983).

In the presence of 100μM GTP, both amylin and CGRP induced the concentration-dependent stimulation of hepatocyte membrane adenylate cyclase activity, where both compounds induced approximately 400 - 600% maximal increases in adenylate cyclase activity. The $E.C._{50}$ values were 0.3 ± 0.14 nM for CGRP and 120 ± 14 nM for amylin (mean ± SEM, n = 5). Treatment of intact hepatocytes with amylin (100 nM) induced a time-dependent increase in intracellular cyclic AMP levels. The antagonist CGRP(8-37) induced a concentration-dependent inhibition of both amylin (100 nM) and CGRP (0.3 nM) -stimulated adenylate cyclase. However, lower concentrations of the antagonist were required to inhibit stimulation by amylin ($I.C._{50}$ = 3.2 nM) compared to CGRP ($I.C._{50}$ = 119 nM). Inclusion of 1 nM CGRP(8-37) produced a 3.9-fold shift to the right of the dose-response curve for amylin ($E.C._{50}$ increased to 468 ± 15 nM) but had no significant effect on the CGRP dose-response curve ($E.C._{50}$ in presence of antagonist = 0.5 ± 0.3 nM). The difference in sensitivity of amylin and CGRP to inhibition by CGRP(8-37) may indicate that these two peptides act through separate receptors to stimulate hepatocyte adenylate cyclase activity.

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206P CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ISOENZYMES (PDE) IN GUINEA-PIG TRACHEAL SMOOTH MUSCLE

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We have compared the elution and inhibition profiles of PDE isoenzymes in guinea-pig trachea (GPT), bovine trachea (BT) and guinea-pig heart (GPH). PDE isoenzymes were separated from the supernatant fraction of homogenised muscle (Elliott et al, 1991), using a Mono Q column on an FPLC system (Pharmacia). Cyclic AMP (1μM) PDE activity was assayed according to the method of Thompson & Appleman (1971).

In each tissue three major elution peaks of activity were qualitatively identified. PDE Ia (inhibited by zaprinast & 10μM cGMP), PDE Ic (Ca/calmodulin stimulated) and PDE II (cGMP stimulated) were common to all three tissues. The third peak of activity was identified as PDE IV (inhibited by Ro 20-1724 but not by cGMP) in BT and PDE III (inhibited by SKF 94120 and cGMP) in GPH. GPT was found, on this basis, to contain both PDE III and PDE IV. The effects of selective isoenzyme inhibitors on the third peak of activity from each tissue and on the spontaneous tone of GPT are shown in Table 1a and b.

(a) Isoenzyme	Bovine Trachea IV	Guinea-pig Trachea III/IV	Guinea-pig Heart III	(b) Effect on spontaneous tone (GPT)
SKF 94120 (PDE III inhibitor)	<50% inhibn at 10^{-3}M	4.72 ± 0.05	5.12 ± 0.05	5.18 ± 0.21
Ro 20-1724 (PDE IV inhibitor)	4.69 ± 0.19	3.52 ± 0.03	3.70 ± 0.08	6.97 ± 0.09
AH 21-132 (Combined III/IV inhibitor)	5.60 ± 0.06	5.41 ± 0.05	6.00 ± 0.07	5.59 ± 0.02

Table 1. (a) Mean (± s.e. mean) values of -log IC_{50} for selective PDE inhibitors tested against third peak of activity obtained from BT, GPT and GPH. Substrate = 1μM cAMP (n=4-6). (b) Mean (± s.e. mean) values of -log EC_{50} for suppression of spontaneous tone of GPT (n=12).

GPT, like canine trachea (Torphy & Cieslinski, 1989), appears to contain both PDE III and IV (Harris et al., 1989; present data). Ro 20 -1724 was much more potent in inhibiting the spontaneous tone of GPT than in inhibiting the isolated III/IV enzyme. This has also been shown by Harris et al. (1989) in GPT with spasmogen-induced tone. By modulating cAMP turnover (possibly within a limited subcellular compartment), PDE IV may play an important role in controlling tracheal tone. Also, Ro 20-1724, like another PDE IV inhibitor, rolipram (Harris et al., 1989), may induce smooth muscle relaxation by additional mechanisms not related to PDE inhibition.

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207P STIMULATION OF *IN VIVO* ACCUMULATION OF INOSITOL MONOPHOSPHATES IN MOUSE CNS BY LITHIUM, PILOCARPINE AND L-658,903

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The assessment of phosphatidyl-inositol (PI) turnover in rodent can be used as a measurement of functional activity for receptors coupled to the PI cycle (Freedman *et al,* 1988). A similar accumulation can be demonstrated *in vivo* using GC detection of inositol monophosphates (Sherman *et al,* 1986). We have adapted the radiometric technique of Margolis and Heller (1966) and the more recent work of Whitworth *et al,* (1990) and prelabelled the phospholipid pool in the CNS of mice by i.c.v. administration of [^{3}H]myo-inositol and examined the effects of lithium and compounds that interact with muscarinic transmission upon the accumulation of inositol monophosphates.

Make BKTO mice (25-30g) were pretreated with [^{3}H]-myo inositol (2μCi/5μl) i.c.v. 24 hours prior to sacrifice. Whole brain was rapidly removed and homogenised in 10 volumes of a chloroform/methanol (1:2) mixture to allow extraction of brain lipids. Following extraction labelled inositol-monophosphates were separated and collected using ion exchange chromatography (Freedman *et al,* 1988) and radioactivity determined using liquid scintillation spectrometry.

In saline treated animals 1.4 ± 0.06% (n = 28) of the total brain radioactivity was present in the inositol monophosphate fraction. Lithium administered s.c. 2 hours prior to sacrifice was able to dose dependently stimulate this accumulation with a MED of 3 meq/kg (2.4 ± 0.14%, n = 6). At a dose of 10 meq/kg this increased to 3.9 ± 0.21%, n = 20). This response was time dependent with a significant response being observed at 1, 2, 3, 18, 24 hours post administration and a peak effect observed at 3 hours. No significant effect was present at 48 hours. The response to 10 meq/kg of lithium was dose dependently stimulated by the muscarinic agonist pilocarpine (MED 10mg/kg) with a maximum response seen at 30mg/kg (9.3 ± 0.8%) and the efficacious oxadiazole agonist L-658,903 at 1 mg/kg (10.4 ± 0.99) (Freedman *et al,* 1990). The peak effect to L-658,903 was inhibited by the muscarinic antagonist scopolamine (1, 10 mg/kg), but not by N-methylscopolamine (1mg/kg).

These results suggest that this model is a useful method to assess the effects of novel muscarinic agents upon phosphatidyl-inositol turnover in the CNS of conscious animals.

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208P INHIBITION OF MUSCARINIC RECEPTOR-STIMULATED INOSITOL POLYPHOSPHATE ACCUMULATION BY LITHIUM IN RAT CEREBRAL CORTEX AND STRIATUM

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There is now substantial evidence that the anti-manic agent lithium can disrupt phosphoinositide signalling in brain by its uncompetitive inhibition of inositol monophosphatase (Berridge et al., 1989). Studies in rat cerebral cortex have demonstrated lithium-induced reductions in the accumulations of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 that appear to result from inositol and polyphosphoinositide depletion (Kennedy et al., 1990). Here we examine the regional specificity of this lithium effect.

Cross-chopped (0.35 x 0.35 mm) cerebral cortex (CC) and striatum (ST) slices were preincubated for 60 min in the absence or presence of [^{3}H]inositol (Kennedy et al., 1990). Packed slices (25 μl) were incubated in 300 μl Krebs-Henseleit buffer equilibrated with O_2:CO_2 (19:1) for 60 min. The time-course of lithium (1 mM) effects and the concentration- dependency (0.1-10 mM) of lithium action was studied in carbachol (CCH)-stimulated slices. Incubations were terminated by addition of trichloroacetic acid (0.5 M) and [^{3}H]InsP_x or mass levels of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 determined as described previously (Kennedy et al., 1990).

Lithium caused concentration-dependent increases in the CCH-stimulated accumulations of [^{3}H]InsP_1 and [^{3}H]InsP_2 in both CC and ST slices with similar lithium concentrations causing half-maximal increases in InsP_1 (EC_{50} 0.5 mM) and InsP_2 (EC_{50} 5 mM), and similar maximal responses in both brain regions. The basal level (expressed as pmol(mg protein)$^{-1}$) of Ins(1,4,5)P_3 in CC (21.5 + 1.0) was greater than in ST (16.3 ± 0.7), but in both regions CCH caused a maximal 40% increase 5 min after agonist addition (CC 29.8 + 1.3; ST 22.6 + 0.6). In both regions Ins(1,3,4,5)P_4 levels were low (CC 3.5 + 0.9; ST 4.1 + 0.6), with maximally CCH-stimulated levels at 5 min being greater in CC (66.4 + 5.0) than in ST (35.6 + 4.7). Analysis of the kinetics of inositol polyphosphate accumulation following CCH stimulation revealed differences between these two brain regions. In particular, both labelling and mass assays demonstrated that unlike CC, ST slices could not sustain maximal Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 accumulations for greater than 5 min. Furthermore, lithium reduced this accumulation with a shorter lag period than that observed for CC. These data suggest that there may be differences in muscarinic receptor-mediated phosphoinositide signalling in these brain regions. Whether this relates to different muscarinic receptor subtypes (M_1/M_3) mediating these responses, or to lower inositol and agonist-sensitive phosphoinositide reserves in ST is not known, but suggests a regional vulnerability to lithium that may have significance to its therapeutic action.

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209P MUSCARINIC RECEPTOR STIMULATION OF PARTICULATE PHOSPHOINOSITIDASE C ACTIVITY FROM CEREBRAL CORTEX

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One of the main transduction pathways for receptor-effector coupling is the activation of phosphoinositide turnover (Berridge & Irvine, 1989). Receptor stimulation of membrane-bound phosphoinositidase C (PIC) activity has been proposed to be mediated through a guanosine triphosphate (GTP) regulatory protein, or G-protein, analogous to G_s - the stimulatory G-protein linked to adenylate cyclase. Evidence for this has derived mainly from studies in particulate fractions demonstrating receptor-stimulated PIC activity with a requirement for GTP or GTP analogues (e.g. Claro *et al.*, 1989). We describe here the GTP analogue dependent, muscarinic receptor stimulation of PIC activity in particulate fractions from mouse and guinea pig cerebral cortex.

A particulate fraction was prepared from mouse or guinea pig cerebral cortical slices pre-labelled with [^{3}H]inositol (120 kBq/mL) for 90 minutes in Krebs Henseleit medium from which $CaCl_2$ was omitted (Alexander *et al.*, 1990). PIC activity was expressed as a conversion from the acidified chloroform-soluble [^{3}H]phosphoinositides to the water-soluble [^{3}H]inositol phosphates, and assessed in at least three separate experiments.

In a particulate fraction from mouse cerebral cortex, increasing the free calcium ion concentration to pCa < 7 led to a significant concentration-dependent increase in PIC activity. At 1 mM free calcium, 12.8 ± 1.0 % of [^{3}H]phosphoinositide was converted to [^{3}H]inositol phosphates. At pCa 6.5, guanosine 5'-(3-thio)-triphosphate (GTP-γ-S) elicited a concentration-dependent increase in PIC activity with an EC_{50} value of 0.6 ± 0.1 μM and a maximal response of 3.1 ± 1.4 %. GTP-γ-S-, but not Ca^{2+}-elicited responses were dependent on the presence of deoxycholate (1 mM) in the incubation medium. The muscarinic agonist carbachol stimulated PIC activity at pCa 6.5 in a GTP-γ-S-dependent (10 μM), atropine-sensitive manner with an EC_{50} value of 46 ± 17 μM and a maximal response of 0.8 ± 0.3 %. Essentially identical results were observed in the guinea pig.

The experiments described are the first demonstration of a receptor-stimulated PIC activity from mouse and guinea pig brain particulate fractions, and are comparable with previous observations in the rat (Claro *et al.*, 1989). The findings support the concept of an intermediate G-protein necessary for muscarinic receptor activation of PIC activity.

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210P COMPARISON OF SOLUBILISED INOSITOL 1,4,5-TRISPHOSPHATE (IP3) RECEPTORS IN BOVINE ADRENAL CORTEX AND RAT CEREBELLUM

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IP3 binding sites in bovine adrenal cortex (BAC) and rat cerebellum (RC) membranes differ in their affinities for IP3 and heparin and their susceptibility to inhibition by Mg^{2+} (Table 1; Varney *et al*, 1990). In the present study we have investigated whether these differences are maintained following solubilisation and purification of the IP3 receptor from both tissues.

Binding of [^{3}H]IP3 to membranes and solubilised receptor was carried out as described by Varney *et al* (1990); reactions were stopped by centrifugation or precipitation with polyethylene glycol, respectively. IP3 receptors were solubilised with 1 % tritonX-100 in the presence of 0.25 M NaCl for 60 min at 4 °C and purified using an heparin-agarose affinity column (Supattapone *et al.*, 1988).

There were marked differences in the susceptibility of IP3 receptors to solubilisation. More than 90 % of IP3 receptors were solubilised in RC by 1 % tritonX-100 while, under the same conditions, less then 20 % were solubilised in BAC. On BAC the affinities of heparin, Mg^{2+} and IP3 were not altered significantly by solubilisation or purification (Table 1). On receptor purified from RC, the ability of Mg^{2+} to inhibit the binding of IP3 was reduced to that seen in BAC while the affinity of IP3 was not altered. The decrease in affinity of Mg^{2+} following purification with heparin-agarose may reflect the loss of calmedin which is present in RC but absent from BAC and confers Ca^{2+}-induced inhibition of IP3 binding in RC (Supattapone *et al.*, 1989).

These results show that Mg^{2+} does not distinguish between purified IP3 receptors in BAC and RC. The differences in affinity of heparin and IP3 for purified receptor in RC relative to BAC may reflect a species difference.

Table 1: IC50s for the displacement of IP3 binding to membranes, tritonX-100 solubilised and heparin-agarose treated preparations of RC and bovine adrenal cortex. Results are from 3 - 5 experiments (S.E. < 15 %).

		membrane	solubilised	heparin-agarose
Bovine adrenal cortex	IP3	7.8 nM	14 nM	14 nM
	Heparin	20 μg/ml	16 μg/ml	18 μg/ml
	Mg^{2+}	9.1 mM	5.0 mM	6.8 mM
Rat cerebellum	IP3	34 nM	25 nM	29 nM
	Heparin	5.5 μg/ml	1.1 μg/ml	1.1 μg/ml
	Mg^{2+}	0.57 mM	0.55 mM	3.0 mM

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211P AUGMENTATION OF ADENOSINE RECEPTOR-STIMULATED CYCLIC AMP ACCUMULATION BY 5-HT IN GUINEA-PIG BRAIN SLICES

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Despite having no effects of their own, certain neurotransmitters or neuromodulators can indirectly potentiate the action of other agents that promote the formation of cyclic AMP in the brain. For instance alpha$_1$-adrenoceptor activation enhances the direct effect of beta-adrenoceptor stimulation in rat cerebral cortex (Robinson and Kendall,1989) and we recently showed that 5HT potentiated the cyclic AMP response to adenosine in guinea-pig brain (Donaldson et al.1990). The identity of the receptor mediating the 5HT effect was not clarified, however, so the purpose of this communication is to pharmacologically characterize this response.

^{3}H-cyclic AMP formation was measured in cross-chopped slices of cerebral cortex from Duncan-Hartley guinea-pigs (either sex) by a ^{3}H-adenine prelabelling method (Donaldson et al.1990). Agonist stimulations were for 10 mins following (when appropriate) 10 mins pre-incubation with antagonists.

The adenosine analogue 2-chloroadenosine (2-CA) at a sub-maximal concentration of 10 μM stimulated ^{3}H-cyclic AMP formation by about 200% (of basal). Despite having no effect alone, co-incubation with 5HT augmented this response in a markedly biphasic manner. The high affinity portion of the dose/response curve had an EC_{50} of 0.8±0.1 μM (n=6) and reached a plateau at 50 μM 5HT. Further increments in 5HT concentration produced large increases in ^{3}H-cyclic AMP production which failed to reach a maximum even at 10 mM 5HT. After 40 mins of pre-incubation with 5HT (50 μM) and a subsequent wash-out, a second challenge with 5HT produced a significantly diminished response with regard to the high affinity phase. However, pre-incubations with 5HT at concentrations up to 3 mM failed to desensitize the low affinity response. Also, the alkylating agent phenoxybenzamine (up to 10 μM) had no effect on the low affinity response despite abolishing the effect of 50 μM 5HT.

DOI ((+)-1-(2,5,dimethoxy-4-iodophenyl)2-amino propane) a putatively selective $5HT_2$ receptor agonist and quipazine both produced small enhancements of the 2-CA response but only at concentrations over 10 μM. A number of other 5HT receptor agonists including 2-methyl-5HT, 5-carboxy-amidotryptamine, 8-OH-DPAT (8-hydroxy-dipropylaminotetraline), mCPP (m-chlorophenylpiperazine), sumatriptan and zacopride all failed to influence either basal or 2-CA-stimulated cyclic AMP formation.

The high affinity portion of the 5HT augmentation was also characterized by incubating slices with increasing concentrations of antagonists in the presence of 50 μM 5HT. The rank order of antagonist affinities was found to be similar to that reported for $5HT_2$ receptor binding in cerebral membranes; K_is (nM) were ritanserin, 0.3; spiperone, 1.9; methysergide, 7.6; cinanserin, 140. Spiroxatrine, pindolol and ICS 205-930 ((3-alpha-tropanyl) 1H-indole-3-carboxylic acid ester) were inactive (up to 10 μM).

The augmentation of 2-CA-stimulated cyclic AMP formation therefore appears to be mediated by a receptor related to the $5HT_2$ but it should be noted that this response differs, particularly with regard to agonist potencies and affinities, from the 5HT-stimulated hydrolysis of inositol phospholipids (Kendall and Nahorski,1985) which is widely regarded as being $5HT_2$ receptor dependent.

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212P ANOMALOUS EFFECTS OF ISOBUTYLMETHYLXANTHINE ON CYCLIC AMP ACCUMULATION IN SH-SY5Y NEUROBLASTOMA CELLS

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Cellular cyclic nucleotide actions are terminated by a number of phosphodiesterase (PDE) isoenzymic activities (Beavo & Reifsnyder, 1990). Although PDE isoenzyme-selective inhibitors have now been described, non-selective agents (such as isobutylmethylxanthine (IBMX)) are still in common use, as selective inhibitors are not yet available for all isoenzymic families (Beavo & Reifsnyder, 1990). Here we report the comparative effects of IBMX and PDE IV-selective inhibitors on cyclic AMP hydrolysis in SH-SY5Y cells.

Cells were cultured in minimal essential medium/10% new-born calf serum. Cells were harvested and washed in Krebs-Henseleit buffer (KHB). Cell suspension (50 μl) was added to 250 μl KHB containing the PDE inhibitors indicated. Forskolin (FSK; 1 μM) was added after 20 min and incubations continued for 10 min. Experiments were terminated and cyclic AMP measured as described previously (Challiss & Nicholson, 1990). PDE inhibitor effects on PDE in cell homogenates were assessed using 1 μM cyclic AMP substrate (Arch & Newsholme, 1976).

IBMX caused >95% inhibition of cyclic AMP hydrolysing activity in homogenates, whereas the PDE IV-selective inhibitors rolipram and Ro 20 1724 caused only 70% inhibition at maximally effective concentrations. However, even at 1 mM, IBMX was much less effective at increasing basal or FSK-stimulated cyclic AMP accumulation compared to the PDE IV-selective inhibitors (see Table).

PDE Inhibitor	Basal	EC_{50} (μM)	Maximal effect (fold-over-basal)	+1 μM forskolin	EC_{50} (μM)	Maximal effect (fold-over-basal)
IBMX		>100	4.2+0.1 (1 mM)		>100	42.0+ 4.6 (1 mM)
rolipram		8.7+2.8	55.3+6.7 (0.1 mM)		4.2+1.3	226.5+37.0 (0.1 mM)
Ro 20 1724		30.0+10.8	22.0+4.5 (0.1 mM)		24.2+4.5	145.0+18.1 (0.1 mM)

Additivity experiments revealed that not only did IBMX exert a poorer than expected effect on cyclic AMP accumulation, but it also potently (IC_{50} 4-10 μM) negated the effects of rolipram both in the absence and presence of FSK. In the presence of IBMX, the potentiating effect of rolipram on FSK-stimulated cyclic AMP accumulation was reduced by 77%. Methylxanthines are well-know for their broad spectrum of activities, including adenosine receptor antagonism, interaction with receptor-G protein-effector coupling (Parsons et al., 1988) and inhibition of cyclic AMP egress from certain cells. We have found no evidence for the presence of adenosine receptors on these cells and have shown that cyclic AMP egress into the incubation medium does not occur to any significant degree. At present we are investigating the possible G protein involvement or unknown secondary locus of IBMX action in these cells.

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213P CHANGES IN HIPPOCAMPAL ADENOSINE AND BACLOFEN SENSITIVITY IN STREPTOZOTOCIN DIABETIC RATS

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In animal models, the diabetic state is accompanied by changes of sensitivity to neurotransmitters and hormones in peripheral tissues. These include alterations of adenosine activity in the heart (Downing, 1985) and adipose tissue (Solomon et al., 1987). Since diabetes is also known to result in behavioural disturbances and changes in central neurochemistry, the present study addressed the question of whether any modification of adenosine and GABA-B receptor function occurred in diabetic rats.

Hippocampal slices 500 μm thick were prepared from male rats. These were preincubated at room temperature before being transferred to the recording chamber where they were superfused with artificial CSF (NaCl 115; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KCl 2; KH_2PO_4 2.2; $NaHCO_3$ 25; glucose 10 mM) gassed with 5% CO_2 in oxygen, at 30°C. A bipolar stimulating electrode was used to stimulate in the stratum radiatum and induce orthodromic population potentials in the CA1 region. Rats were made diabetic by injecting streptozotocin (60 mg/kg i.p.). They were killed and slices prepared 3-14 days later.

Both adenosine and baclofen induced a dose-dependent reduction in the amplitude of orthodromic potentials in slices from control animals. The ED_{50} values were 27.71 ± 5.44 (s.e.m.) μM (n = 8) for adenosine and 2.3 ± 0.2 μM (10) for baclofen. In diabetic rats the ED_{50} value for adenosine shifted to 6.58 ± 1.0 μM (9), ($P<0.005$) and that for baclofen to 1.3 ± 0.3 μM (10). The greater potency of adenosine was not shown by 2-chloroadenosine for which the ED_{50} concentration was increased from 0.47 ± 0.08 (3) to 0.89 ± 0.44 μM ($P<0.01$).

In order to determine the mechanism of these effects adenosine was again tested in the presence of the transport inhibitor hydroxy-nitrobenzylthioguanosine (HNBTG, 10 μM). In control slices this reduced the ED_{50} concentration to 11.0 ± 2.06 (5), comparable with that seen in diabetic animals. When HNBTG was included in tests of the diabetic hippocampi the adenosine ED_{50} of 6.25 ± 1.05 μM (5) was not significantly different from that obtained in the absence of HNBTG.

This work suggests that the adenosine transporter is absent or inactive in the diabetic hippocampus, resulting in an increased potency of adenosine. There is also a decreased potency of 2-chloroadenosine, possibly reflecting a down-regulation of receptor function. The mechanisms underlying these changes of adenosine and baclofen sensitivity remain to be explored, though preliminary experiments indicate that acute exposure to glucocorticoids or ketones are not involved.

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214P OPERANT "BEHAVIOURAL DEPENDENCE" STUDIES WITH CHLORDIAZEPOXIDE IN RATS: LACK OF EFFECT OF ONDANSETRON

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Chronic administration of many drugs prior to repeated operant sessions typically leads to tolerance, and to disruption of behaviour on withdrawal. Such behavioural disruption is termed "behavioural dependence" (Schuster & Thompson, 1969), and is thought to be a sensitive assay for withdrawal, since drugs such as cocaine which do not induce a gross withdrawal syndrome induce "behavioural dependence" (Woolverton & Kleven, 1988). This study assessed: (i) The ability of chronic chlordiazepoxide (CDP) treatment to induce "behavioural dependence", and (ii) Effects of chronic treatment with the 5-HT3 antagonist ondansetron on such "behavioural dependence". Female rats were trained on a food rewarded FR20 operant schedule. Subsequently, they were treated chronically with saline (controls) or CDP. The initial dose of CDP was 10 mg/kg/day. This suppressed responding by c. 40%. Tolerance developed rapidly to this effect (3 days). The dose was therefore increased to 20 mg/kg/day, which again suppressed responding by c. 40%. Tolerance developed to this effect after 14 days. The dose was increased to 30 mg/kg/day, which again suppressed responding. After 25 days treatment with this dose (a total of 42 days treatment in all), withdrawal was initiated. Animals were treated chronically b.i.d. with saline or ondansetron at either 0.1 or 0.01 mg/kg, these ondansetron dosing regimes having been reported to alleviate other behavioural signs of benzodiazepine (BZ) withdrawal (Costall et al. 1989; Goudie and Leathley, 1990). In saline treated animals there was a marked (30%) significant initial suppression of responding during withdrawal ($t = 5.9$, $df = 8$, $p < 0.01$), which recovered over a number of days of withdrawal. Similar initial withdrawal signs were seen in both groups of ondansetron treated subjects (smallest $t = 2.8$, $df = 7$, $p < 0.03$). Ondansetron did not alleviate the observed BZ withdrawal signs ($F = < 1.0$, NS). These data show that it is possible to demonstrate "behavioural dependence" with a benzodiazepine, and that ondansetron fails to attenuate this specific BZ withdrawal sign, in contrast to its positive effects against other withdrawal signs (Costall et al. 1989; Goudie & Leathley, 1990). These data therefore suggest that ondansetron may have limited utility in the treatment of BZ withdrawal.

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215P DIFFERENTIAL ACUTE ACTION OF SODIUM VALPROATE, VIGABATRIN, BECLAMIDE AND AMINOBECLAMIDE ON 5-HTP-INDUCED HEAD TWITCH

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It has been established that administration of 5-hydroxy-L-tryptophan (5-HTP) produces characteristic head twitch behaviour in mice which is differentially affected by agents which modify central GABA transmission (Handley and Singh, 1985). In this context, sub-chronic (14 day) treatment with the anticonvulsant sodium valproate increases this head twitch response (Green et al. 1985). We now compare the acute effect of the following anticonvulsants possessing inhibitory activity on GABA metabolising enzymes as one of their component mechanisms: sodium valproate (Chapman et al. 1982), vigabatrin (Jung et al. 1977), beclamide (Sawaya et al. 1975) and its analogue N-(p-aminobenzyl)-β-chlorpropionamide (aminobeclamide) on 5-HTP-induced head twitch.

Male ICI-GB1 mice (30±5g) were habituated (60 min) prior to testing then given carbidopa (25mg kg^{-1}sc) followed by 5-HTP (100mg kg^{-1} ip) 15 mins later. The incidence of head twitch was recorded for paired animals 20 mins following 5-HTP administration for a duration of 5 mins. Sodium valproate (ip), vigabatrin (ip), beclamide and aminobeclamide (po) were introduced respectively 30, 360, 45 and 45 mins before head twitch counting.

Vehicle pretreated animals yielded overall mean control head twitch counts of 20.3±1.3. Sodium valproate dose-dependently (50-300mg kg^{-1}) inhibited head twitch expressed as % control by values which ranged from 48.3 to 80.8% ($P<0.001$) and similarly, vigabatrin (200-600mg kg^{-1}) evoked graded reductions in head twitch (32.0-63.1%; $P<0.005$). In contrast, beclamide (100-250mg kg^{-1}) potentiated the head twitch response (37.5-54.5%; $P<0.01$) and a comparable effect was noted for aminobeclamide (50-150mg kg^{-1}) which augmented control values by 35.0-71.5% ($P<0.01$).

Since sodium valproate and vigabatrin ultimately elevate central GABA levels through inhibition of GABA-T and other mechanisms, it is probable that the resultant increase in GABA function is responsible for head twitch attenuation (Handley and Singh, 1985). Although beclamide inhibits GABA metabolism, it facilitated head twitch and this action may arise from an acute depletion of central 5-HT (Darmani et al. 1991) which would transiently increase intrasynaptic 5-HT. Since aminobeclamide possessed similar head twitch effects, it is proposed that it may share a related underlying mechanism.

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216P FLUMAZENIL ANTAGONISES THE ANXIOLYTIC EFFECT OF CHLORDIAZEPOXIDE, BUSPIRONE AND DOTHIEPIN IN THE ELEVATED PLUS-MAZE

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The benzodiazepine antagonist flumazenil attenuates the anxiolytic effect of chlordiazepoxide in the elevated plus-maze model of anxiety (File, 1990). Non-benzodiazepine drugs such as buspirone (Dunn et al., 1989; Söderpalm et al., 1989) and dothiepin (Luscombe et al., 1990) also exhibit anxiolytic activity in this model. We have therefore compared the effect of flumazenil on the anxiolytic effects of chordiazepoxide, buspirone and dothiepin in the elevated plus-maze.

Male CD rats (160 - 200g; Charles River) were orally administered either deionised water or chlordiazepoxide (3 mg/kg), buspirone (0.1 mg/kg) or dothiepin (3 mg/kg) at doses previously shown to induce a similar anxiolytic effect (see Table 1). After 30 min the animals were injected with either flumazenil (4 mg/kg ip) or saline. Thirty minutes later the rats were placed in the centre of an elevated plus-maze and the number of open and closed arm entries counted during 10 min (Luscombe et al., 1990).

Flumazenil similarly antagonised the anxiolytic effects of chlordiazepoxide, buspirone and dothiepin, attenuating the anxiolytic-induced increase in the open:closed arm entries ratio (Table 1). Flumazenil reversed the increase in total arm entries caused by buspirone and dothiepin, but not the increase caused by chlordiazepoxide. Flumazenil alone did not alter either the open:closed arm entry ratio or the total number of arm entries (Table 1).

Table 1 Effect of flumazenil on the anxiolytic activity of chordiazepoxide, buspirone and dothiepin in the elevated plus-maze

Pretreatment	Treatment	Open:closed arm entries	Total entries	Treatment	Open:closed arm entries	Total entries
Water	Saline	0.027 ± 0.017	16.3 ± 0.6	Flumazenil	0.016 ± 0.014	16.0 ± 0.3
Chlordiazepoxide	Saline	0.322 ± 0.012**	19.2 ± 0.8**	Flumazenil	0.198 ± 0.010††	18.5 ± 0.7
Buspirone	Saline	0.313 ± 0.015**	19.7 ± 0.5**	Flumazenil	0.193 ± 0.011††	16.5 ± 0.7††
Dothiepin	Saline	0.318 ± 0.008**	19.3 ± 0.7**	Flumazenil	0.206 ± 0.013††	16.8 ± 1.1†

Values are mean ± SEM (n=6). ** $p < 0.01$ vs water + saline (Dunnett's test); †† $p < 0.01$, † $p < 0.05$ vs drug + saline (multiple t-test).

The similar activity of flumazenil against the anxiolytic effect of the three drugs on the open:closed arm entries ratio suggests that they may act through a final common pathway although it is unlikely to be directly through the benzodiazepine receptor since buspirone and dothiepin have only negligible affinity for this receptor.

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217P THE EFFECT OF GUANOSINE 3,5,-CYCLIC MONOPHOSPHATE UPON SECRETAGOGUE AND CALCIUM-EVOKED ACTH SECRETION FROM AtT-20 PITUITARY TUMOUR CELLS

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Guanosine 3'5'-cyclic monophosphate (cyclic GMP) may mediate atriopeptin-evoked inhibition of ACTH secretion from pituitary corticotrophs (Antoni & Dayanithi, 1989). This was investigated in the ACTH-secreting AtT-20 anterior pituitary tumour cell line by measuring the ability of (a) agents which elevate cellular cyclic GMP content to inhibit corticotrophin-releasing factor (CRF)-stimulated ACTH secretion and (b) cyclic GMP to inhibit calcium-evoked ACTH secretion from electrically permeabilised AtT-20 cells.

The effect of atriopeptin, 8 bromo cyclic GMP (8BrcGMP) and sodium nitroprusside (SNP) upon CRF-stimulated ACTH secretion from intact cells was studied using methods previously described (Hook et al, 1982). The effect of cyclic GMP upon calcium-evoked ACTH secretion from electrically permeabilised AtT-20 cells was investigated using methods previously described (Guild et al., 1988). ACTH levels were estimated by radio-immunoassay (Reisine, 1984).

CRF (10^{-12}-10^{-7}M) stimulated a concentration-dependent (EC_{50} = 10^{-9}M) increase in ACTH secretion which was unaffected by atriopeptin (10^{-10}M) or 8BrcGMP (10^{-5}M). No effect of atriopeptin (10^{-11}-10^{-7}M), 8BrcGMP (10^{-8}-10^{-4}M) or SNP (10^{-9}-10^{-5}M) upon CRF (10^{-9}M)-stimulated ACTH secretion was observed. Calcium (10^{-9}-10^{-3}M) stimulated a concentration-dependent (EC_{50} = 10^{-6}M) increase in ACTH secretion from electrically permeabilised cells which was unaffected by cyclic GMP (10^{-5}M). Cyclic GMP (10^{-8}-10^{-4}M) did not affect calcium (10^{-5}M)-stimulated ACTH secretion at any concentration studied.

No evidence was obtained demonstrating inhibition of ACTH secretion from AtT-20 cells by agents which elevate cellular cyclic GMP content. This may reveal another difference between the AtT-20 tumour cell line and the normal corticotroph. In addition, the ability of cyclic AMP to potentiate calcium-evoked ACTH secretion (Guild et al., 1988) is not mirrored in the ability of cyclic GMP to inhibit calcium-evoked secretion from electrically permeabilised AtT-20 cells.

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218P ENDOTOXIN AND THE EXPRESSION OF LIPOCORTINS IN CENTRAL AND PERIPHERAL TISSUES OF THE RAT

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The lipocortins (LCs) comprise a group of structurally related Ca^{++} and phospholipid binding proteins. LC I was first described as a steroid inducible protein but it is now apparent that other blood-borne factors may also influence its expression . In this study we have examined the effects of endotoxin, which liberates e.g cytokines from macrophages and produces a sustained elevation in pituitary-adrenocortical activity,on the expression of LCs I,II & V in the adenohypophysis, hypothalamus, lung and thymus of chronically (> 14 days) adrenalectomized and sham-operated rats.Groups (n=4-5) of male Sprague-Dawley rats (~300g) were injected either with endotoxin (250µg/kg, i.p) or an equal volume of the saline vehicle (1.0 ml/kg, i.p). They were killed 0, 1, or 3h later and the relevant tissues removed rapidly and stored at -70° C. The LC I, II and V contents of protein extracts of the tissues were estimated by western blotting. LC I was also determined by competitive ELISA (Smith et al., 1990). LCs I, II & V were readily dectectable in all tissues examined.Endotoxin produced ,within 3h, significant ($P<0.05$) increases in the LC I content of adenohypophysial tissue from sham operated (vehicle: 697 ± 279 vs endotoxin :1183 ± 335, arbitrary units) but not adrenalectomized rats. In contrast, it had no effect on the expression of the protein in hypothalamic or thymic tissue from either group but greatly increased the LC I content in the lung of adrenalectomized (control: 34 ±10; endotoxin 1h: 312 ± 92; endotoxin 3h: 205± 44) but not sham-operated rats. Similar LC I profiles were observed when the protein was determined by ELISA. LC II levels were also significantly elevated in the lungs of adrenalectomized but not sham-operated animals 1 and 3h after the injection of endotoxin (control:146 ± 18; endotoxin 1h: 295 ± 36; 3h: 258 ± 22). However, there were no discernable, endotoxin-induced changes in the LC II contents of the other tissues examined. Furthermore, the expression of LC V in tissues from adrenalectomized and sham-operated rats was unaffected by the toxin.The results indicate that endotoxin induces LC I in the adenohypophysis and LCs I & II in the lung. Its actions on the adenohypophysis appear to depend on the glucocorticoids. Those in the lung are evident only in adrenalectomized animals and may therefore be effected by mediators such as cytokines whose release/activity is attenuated by glucocorticoids.

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219P THE EFFECT OF AGEING ON STONE MAZE PERFORMANCE AND BRAIN LEVELS OF CHOLINE ACETYLTRANSFERASE

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Cognitive deficits have been previously documented in aged animals using appropriate tests of memory such as the radial maze (Ingram et al,1981). These deficits have been attributed to degeneration of the neurones of the cholinergic forebrain (McGeer et al,1984). This study used the Stone maze (Stone,1929) to investigate the effect of age upon the performance of a complex reference memory task and this was correlated with levels of the presynaptic marker enzyme choline acetyltransferase (ChAT) in 8 brain areas.

Male Lister hooded rats allocated to 3 age groups; 4 months (200-250g), 12 months (600-750g), and 20 months (600-750g) (n = 8), maintained at 85% of body weight were trained on a 9 day protocol for a food reward on the Stone maze, as previously described (Bratt et al., 1990). Each animal was given one 5 min trial per day in the maze. The time taken to complete the maze (latency s) and the number of errors made were recorded.

Performance of the maze was severely impaired at age 12, and 20 months, as compared to young animals. Latency (s) and number of errors (expressed ±SEM) were significantly increased between days 1-9 for 12 and 20 month old rats as compared to young animals. For example on day 9 of training, latency was increased from 14.7±0.88s (3 months) to 100.58±12.78s** (12 months) and 122.0±26.48s** (20 months). The number of errors were increased from 2.1±0.23 (3 months) to 4.42±0.51* (12 months) and 4.67±0.67* (20 months) (*P<0.05, **P<0.01, ANOVA followed by Dunnett's t-test).

ChAT levels were measured in the frontal, temporal and entorhinal cortices, amygdala, hippocampus, septum, striatum, and tuberculum olfactorium, using the method of Fonnum (1975). A reduction in ChAT occurred in the frontal and temporal cortices alone, of aged animals and young naïve animals, which had not been exposed to the maze as compared to young trained rats. However, in the temporal cortex, this failed to reach significance.

Table 1: The effects of age and training on ChAT levels (nmol/min/mg protein) ± SEM

	Untrained 3 months	Trained 3 months	Untrained 20 months	Trained 20 months
Frontal cortex	49.77±13.79	213.89±69.75	50.27±7.95*	43.86±6.33*
Temporal cortex	52.26±3.92	105.6 ±28.29	67.43±7.51	59.58±5.97

(*P<0.05, Student's t-test)

These results suggest that a decrease in cortical cholinergic function may occur as a result of the ageing process, and this could contribute to the observed memory dysfunction.

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220P DISTRIBUTION OF THYROTROPHIN-RELEASING HORMONE-LIKE IMMUNOREACTIVITY AND CHOLINE ACETYLTRANSFERASE ACTIVITY IN THE GUINEA-PIG BRAIN

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The present study examined the regional distribution of choline acetyltransferase (ChAT) activity and thyrotrophin-releasing hormone (TRH)-like immunoreactivity (LI) in the guinea-pig brain and spinal cord.

Male Dunkin-Hartley guinea-pigs (300 - 400 g) were killed by cervical dislocation and decapitation and the brain and spinal cord rapidly removed and dissected on a cool tray (4°C) into frontal cortex (FC), hypothalamus (Hyp), hippocampus (Hip), septum (Sep), midbrain (MB), brainstem (BS), cerebellum (CBM), globus pallidus (GP) and dorsal (DSC, third) and ventral (VSC, two thirds) portions of the thoracic spinal cord, which were stored at -60°C prior to assay. TRH was extracted (sonication for 10 sec in 45 % v/v methanol : 45 % v/v 0.1 M acetic acid : 10 % v/v 1.05 M aqueous sodium metabisulphite) and levels determined in triplicate from the linear portion of the standard curve using a sensitive and specific radioimmunoassay and a polyclonal sheep antiserum. ChAT activity was determined in triplicate following homogenisation and incubation (for 30 and 10 min at 25°C in a 0.1 M phosphate buffer, pH 7.5, containing 150 µM eserine, 28 µM choline iodide and 0.4 % v/v Triton X100) using a radioenzymatic assay (see Fone *et al.*, 1988 for further details). Recovery of exogenous TRH from guinea-pig cortex was 58 ± 8 %, the limit of detection being 5 pg/tube.

TRH-LI levels were greatest in Hyp but high levels were also present in Sep with moderate levels in BS and VSC (Table 1). ChAT activity in the SEP and GP (969±345) was larger than that detected in any other region while that in the CBM was virtually undetectable (Table 1).

Table 1 TRH-LI (pg/mg protein) and ChAT (nmol Ach/h/mg wet weight) in guinea-pig brain (mean ± s.e.mean, n = 6-10)

Region	FC	Hyp	Hip	Sep	MB	BS	CBM	DSC	VSC
TRH	4±1	2448±416	40±8	1248±427	66±7	200±31	12±4	127±26	234±59
ChAT	284±106	277±55	327±24	859±111	161±44	112±18	16±4	133±18	242±26

The distribution of TRH-LI in the guinea-pig brain is analogous to that previously reported in the rat (Lighton *et al.*, 1984) but shows little relationship to the TRH receptor density in these regions (Taylor & Burt, 1982). Similarly the distribution of ChAT activity agrees well with previous immunocytochemical studies in the rat (Houser *et al.*, 1983).

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221P DISTRIBUTION AND RELEASE OF ACETYLCHOLINE IN RAT BRAIN MEASURED BY HPLC WITH ELECTROCHEMICAL DETECTION

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HPLC with electrochemical detection (ED) is a recent sensitive method for acetylcholine (ACh) measurement in which the separation of ACh and choline (Ch) by reverse phase HPLC is followed by the detection of hydrogen peroxide produced enzymatically from acetylcholinesterase (AChE) and Ch oxidase. In this study HPLC-ED coupled with a modification of the extraction procedure of Eva *et al*.(1984) was developed to (a) measure ACh and Ch levels in selected brain regions, and (b) determine the *in vitro* release of ACh from incubated tissue slices. For the determination of regional ACh and Ch levels, male Wistar and Hooded Lister rats (200g) were exposed to microwave irradiation (2.45GHz, 2.4KW, 6s), decapitated, brain regions dissected, frozen and stored at -60°C. ACh and Ch were extracted in 0.4M perchloric acid, precipitated with 5mM tetraethylammonium and 2%v/v Reineckate and re-eluted in mobile phase (pH 7.0), using ethylhomocholine (EHC) as an internal standard (percentage recovery was EHC 24.5±16.9%, ACh 20.5±6.4% and Ch 20.9±8.4%, n=5). In release experiments Hooded Lister rats (200-250g) were killed by cervical dislocation, striatal tissue removed, sliced and incubated at 37°C in Krebs containing Ch (2μM) and neostigmine (3.8μM) and the effects of depolarising levels of K^+ investigated using the fixed volume method of Bennett *et al*.(1983). Striatal levels of ACh were twice, four, and five times those of cortex, septum and hippocampus, respectively. In hippocampus there was twice as much Ch as ACh, while in cortex the ACh and Ch levels were equal, implying different turnover in these regions. Striatal Ch levels were twice those of cortex and hippocampus respectively.

Table 1 ACh and Ch levels in selected brain regions, nmol/mg protein (mean±s.e.m, n=5-6)

		Striatum	Cortex	Septum		Hippocampus
ACh	(Wistars)	0.52±0.16	0.25±0.04	0.14±0.05	(Hooded Listers)	0.10±0.02
Ch		0.39±0.14	0.23±0.06	not detected		0.18±0.03

High K^+ (50mM, n=5) tended to elevate ACh release into supernatants of striatal slices (0.66±0.06 nmol/mg protein compared to control, 0.44±0.05, not significant), and simultaneously produced a significant decrease in the ACh content of the slices (0.17±0.04 compared to control 0.43±0.10, $p<0.05$, Student's t-test). In separate experiments K^+ (25mM) evoked release of ACh from striatal slices was Ca^{2+} dependent (Ca^{2+} free, 4.39±0.24 and 1.25mM Ca^{2+}, 20.01±6.3 nmol/g wet wt., $p<0.05$, Student's t-test, n=4). The present rat brain ACh levels are comparable to those previously reported using HPLC-ED, (Potter *et al.*,1983) or chemiluminescent detection, (Wetherall *et al.*, 1983). Furthermore this study demonstrates the measurement of endogenous ACh release *in vitro*. Subsequent studies will determine the effect of drugs on ACh release with particular reference to agents which may be used in the treatment of Alzheimer's disease.

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222P PHARMACOLOGICAL CHARACTERISATION OF MUSCARINIC RECEPTORS AND CHOLINE REUPTAKE SITES IN INTRACEREBRAL IMPLANTS OF FOETAL RAT BASAL FOREBRAIN

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Intracerebral implants of foetal neuronal tissues can restore many of the hormonal, metabolic and behavioural deficits associated with specific lesions within the central nervous system (Dunnett & Richards, 1990). These restorative effects appear to be associated with the outgrowth of neuronal processes from the implant into the host brain. Whilst there is abundant evidence that implanted neurons contain appropriate neurotransmitters (Dunnett & Richards, 1990), it is unclear whether these neurons also possess normal receptors and reuptake mechanisms. In the present study we have examined the binding characteristics of cholinergic muscarinic receptors and choline reuptake mechanisms in intracerebral implants derived from foetal basal forebrain.

Unilateral ibotenate-induced lesions of the basal forebrain were produced in 15 male Sprague-Dawley rats, followed 8-10 days later by the intracerebral implantation of cell suspensions derived from foetal rats (gestational age, 16days) (Sharkey *et al.*, 1991). Approximately 12 weeks after implantation, the animals were sacrificed and the brains sectioned and processed for radioligand binding autoradiography (Dawson *et al.*, 1991). Transplants were identified in adjacent sections by acetylcholinesterase histochemistry. Muscarinic receptors and choline reuptake sites were examined using [^{3}H]-quinnuclidinyl benzylate (QNB) and [^{3}H]-hemicholinium-3 (HC3) binding autoradiography respectively. M1 and M2 components of [^{3}H]-QNB binding were differentiated by pirenzepine competition.

Unilateral lesions of the nucleus basalis reduced both [^{3}H]-QNB (-25%) and [^{3}H]-HC3 (-43%) binding throughout host fronto-parietal cortex ipsilateral to the lesion but no significant changes were detected elsewhere. Saturation analysis of [^{3}H]-QNB and [^{3}H]-HC3 binding within the implanted tissue revealed the presence of a single population of binding sites with affinities similar to that of the host tissue (0.43nM and 20.4nM respectively). The density of [^{3}H]-QNB and [^{3}H]-HC3 binding within the implant appeared to be independent of the site of implantation, remaining constant irrespective of whether the transplant was sited in cortical grey or subcortical white matter. Competition analysis revealed the presence of two distinct populations of [^{3}H]-QNB labelled binding sites within the transplant: a high affinity site (K_H = 6nM) and a low affinity site (K_L = 492nM). The ratio of high to low affinity sites within the transplant was not significantly different from that of the intact contralateral cortex, but was different from that of the host basal forebrain where muscarinic receptors are almost exclusively of the M2 subtype. In conclusion it would appear that although implants develop receptors which are pharmacologically similar to that observed in the adult brain the receptor profile which develops in the implant appears to be independent of the site of implantation and is dissimilar to that which would ultimately develop in the donor tissue.

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223P INTERACTION OF CERTAIN CALCIUM ANTAGONISTS WITH SIGMA BINDING SITES

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Calcium antagonists have been reported to modulate PCP receptor interactions. In particular, verapamil potently displaces [^{3}H] PCP binding in rat brain (Quirion & Pert., 1982). Given the close relationship between PCP and σ ligands we have compared the affinity of verapamil, together with a variety of other calcium antagonists, for the σ binding site.

Brains from male Dunkin-Hartley guinea-pig were homogenised and a frozen membrane suspension prepared as previously described (Fox et al., 1991). Samples were incubated for 90 minutes at room temperature in a final volume of 1 ml 50 mM Tris-HCl (pH 7.4) containing 3.4 nM [^{3}H] DTG, unlabelled drugs and membrane (1 mg protein). Non-specific binding was defined as that remaining in the presence of 10 μM haloperidol.

Compound	IC_{50} (nM)		
Prenylamine	25.0±3.4	Nifedipine	> 10,000
Pimozide	271.3±9.9	Nitrendipine	> 10,000
Verapamil	546.7±162.5	Diltiazem	> 10,000
Bepridil	135.8±12.9	Haloperidol	3.7±0.9
Fendiline	167.3±40.1	DTG	36.3±14.8
Calmidazolium	>10,000	PCP	1419.2±186.6
D600	4741.5±1204.4	Pentazocine	89.4±24.3

Results are expressed as mean ± s.e. mean from at least 3 experiments conducted in triplicate unless otherwise indicated.

Certain calcium antagonists such as prenylamine, pimozide and verapamil show high affinity for the σ binding site, whilst diltiazem and the dihydropyridines are essentially inactive at concentrations up to 10 μM. A similar relationship between these compounds was also found against [^{3}H] dextromethorphan binding (Klein & Musaccio, 1989).

It is intriguing to speculate upon the contribution made by the interaction of calcium antagonists with the sigma binding site towards the suggestion from limited clinical trials that certain calcium antagonists possess antipsychotic properties. In particular, antipsychotic effects have only been demonstrated by non-dihydropyridine Ca^{2+} antagonists such as verapamil and pimozide.

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224P POTENTIATION OF OPIOID ANTINOCICEPTIVE ACTIVITY BY EPHEDRINE AND PHENYLPROPANOLAMINE IN MICE

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Ephedrine and phenylpropanolamine (PPA) are commonly used in combination with opioids in "cold remedies" or "cough mixtures". These preparations are freely available over the counter in most countries. Both ephedrine and PPA are known to have biochemical central nervous system effects qualitatively similar to, though quantitatively less than, amphetamine (Dietz et al., 1985). In view of the recent observation in Hong Kong (Chan et al., 1990) of increasing abuse of proprietary formulations of "cough remedies" containing ephedrine and codeine, the present study was undertaken to study the effects of ephedrine and phenylpropanolamine on the antinociceptive effect of both morphine and codeine in mice.

Male ICR mice weighing between 30-35g were used. Antinociceptive effect was measured by the tail flick method. The ED_{50} value of morphine or codeine after the various pretreatments was considered significantly different from the control value if it fell outside the 95% confidence limits of the latter, and also if the control ED_{50} lay outside the 95% confidence limits of the experimental ED_{50}.

When given alone, ephedrine and PPA had no antinociceptive effects in the doses employed in the present study. Ephedrine and PPA dose dependently potentiated the antinociceptive effects of morphine 5.0mg/kg and codeine 60.0mg/kg. These doses of morphine and codeine were approximately the ED_{50} values of the respective drug in naive animals. Apart from the effect on single-dose antinociceptive effects of codeine and morphine, the effects of ephedrine and PPA on the ED_{50} values of these two opioid drugs were also studied. Ephedrine and PPA both caused a parallel shift of the dose-response curves of morphine to the left. Ephedrine dose-dependently enhanced the antinociceptive effect of morphine and the ED_{50} values in these pretreated groups were significantly lower than saline pretreated control animals. PPA also enhanced the antinociceptive effect of morphine though the dose-response relationship was not as clearcut as for ephedrine. Similar potentiating effect of these sympathomimetics was also observed with codeine antinociceptive activity.

In the present study, the shifts in the dose-response curves were parallel in all cases suggesting that the basic mechanism of antinociceptive effect of the opioids was not altered by pretreatment with sympathomimetics. Despite various pretreatment with sympathomimetics, the peak antinociceptive effects of codeine and morphine were not altered indicating that pharmacokinetic changes are unlikely to be responsible for the observed enhancement of opioid antinociceptive activities. The present findings that ephedrine and PPA potentiate the acute antinociceptive effects of codeine and morphine may be a clue to the high abuse rate. It is possible that the mixture is more reinforcing than codeine alone, and so is more liable to be abused.

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225P OPIOID ACTIVITY OF SOME ANALOGUES OF DERMENKEPHALIN AND DELTORPHIN I IN BINDING ASSAYS AND BIOASSAYS

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Dermenkephalin (Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2) and deltorphin I (Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2) are two amphibian peptides isolated from the skin of *Phyllomedusae* which have high affinity and selectivity for δ-opioid binding sites (Amiche *et al*, 1989; Erspamer *et al*, 1989). In order to elucidate the structural features required for these δ-actions, we have determined, in binding assays and in isolated tissue bioassays, the opioid activity of a series of dermenkephalin and deltorphin I analogues. Binding to μ-, δ- and k-sites in homogenates of rat brain was carried out as described previously (Amiche *et al*, 1989). The analogues were tested for their effectiveness in depressing the electrically-evoked contractions of the vas deferens of the hamster (δ-opioid receptors) and the guinea-pig myenteric plexus preparation (μ- and k-opioid receptors). All bioassays were carried out in the presence of a combination of peptidase inhibitors (McKnight *et al*, 1985).

Dermenkephalin and deltorphin I had very low agonist activities in the myenteric plexus preparation (IC_{50} values > 3000nM). In contrast, they were much more active at the δ-receptors in the hamster vas deferens where the IC_{50} values were 7nM and 3.6 nM, respectively. Interestingly, in the hamster vas deferens the potency of dermenkephalin was increased 39-fold following inhibition of tissue peptidases whereas, the potency of deltorphin I was unchanged. In the hamster vas deferens, deltorphin-(1-6)-NH_2 had an IC_{50} value of only 278nM; further shortening of the peptide to deltorphin-(1-4)-NH_2 resulted in a complete loss of δ-activity. Similar findings were obtained with dermenkephalin-(1-6)-NH_2 and dermenkephalin-(1-4)-NH_2.

The data from the binding assays confirm those of the bioassays: dermenkephalin binds with high affinity and selectivity to δ-binding sites (K_i at μ-sites 267nM, δ-sites 5.3nM and k-sites >25,000nM) as does deltorphin I (K_i at μ-sites 200nM, δ-sites 5.8nM and k-sites >25,000nM). Furthermore, progressive shortening of both peptides resulted in analogues devoid of signicant affinity for the δ-binding site.

These results show that although the C-terminal tetrapeptides of dermenkephalin and deltorphin I differ in their amino acid composition, they may play similar roles in specifying the correct addressing of these peptides for the δ-opioid receptor.

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226P PHARMACOLOGICAL CHARACTERISATION OF ANGIOTENSIN II-INDUCED DEPOLARISATION OF THE RAT ISOLATED SUPERIOR CERVICAL GANGLION

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Angiotensin II (AII) receptors have been separated into two subtypes, AT_1 and AT_2 (Bumpus et al, 1991), based on the reciprocal selectivity of the nonpeptide antagonists, DuP 753 and EXP655 respectively (Chiu et al, 1989). In addition, dithiothreitol (DTT) inhibits radioligand binding to AT_1 sites (Whitebread et al, 1989) and abolishes AT_1-mediated functional responses in rabbit aorta (Fleisch et al, 1973). We have previously reported that AII depolarises rat isolated superior cervical ganglia (SCG) (Hawcock et al, 1991) and the aim of the present study was to characterise these responses using DTT and the selective AII receptor antagonists.

Superior cervical ganglia from male LH rats (300-350g) were desheathed and arranged in two compartment chambers. The ganglion cell body was separated from the post-ganglionic trunk by a grease barrier. Both regions were perfused with Krebs buffer at 37°C, bacitracin (1mM) being included in the buffer to inhibit the metabolism of AII. Potential differences (mV) across the grease barrier were recorded using Ag/AgCl electrodes. To avoid tachyphylaxis, increasing concentrations of AII were applied serially for 5 min with a 20 min interdose interval. Antagonists were incubated for 30 min prior to re-establishing the AII concentration-response curve. Where appropriate, antagonist potency was expressed as estimates of mean pK_B (with 95% confidence limits), obtained from Schild analysis.

DuP 753 produced parallel rightward displacement of the AII concentration-response curve. The mean pK_B was 8.28 (8.12-8.43) and slope of the Schild plot was 1.15±0.13 (not significantly different from 1). Preincubation of the SCG with 3μM EXP655 failed to antagonise the AII-induced depolarisation. DTT (1mM) failed to modify the depolarising responses to concentrations of AII up to 0.01μM, but partially reduced responses to higher agonist concentrations (maximally by 22±5.9%).

The ability of DuP 753 but not EXP655 to antagonise the AII-induced depolarisations of rat SCG suggests that these responses are mediated by the AT_1 subtype. The inability of DTT to completely inhibit AT_1-mediated responses in this tissue contrasts with its inability to inhibit AT_1-mediated responses in rabbit aorta, although Barnes et al (1991) also report that DTT fails to inhibit AT_1-mediated contractions of rat uterus. The apparent selectivity of DTT to modify AT_1-mediated functional responses in some tissues but not in others requires further investigation.

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227P ANTIBODY MICROPROBES DETECT IMMUNOREACTIVE SUBSTANCE P OVER AN INCREASED AREA OF THE DORSAL HORN FOLLOWING ENDOPEPTIDASE INHIBITION

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When detected with antibody microprobes, the tachykinins substance P (SP) and neurokinin A (NKA) show very different patterns of release in the spinal cord following a peripheral noxious stimulus. Release of immunoreactive (ir)SP is centered on the substantia gelatinosa whereas irNKA is detected diffusely over the dorsal horn. As these tackykinins have similar sites of termination in the dorsal horn it was proposed that NKA is relatively resistant to degradation and hence diffused away from sites of release. The present experiments have examined whether protecting SP from degradation causes this neuropeptide also to access sites remote from sites of release.

Antibody microprobes were prepared by coating glass micropipettes with a siloxane polymer bearing free amine groups and antibodies were immobilized to this layer through glutaraldehyde and protein A. They were inserted into the spinal cords of barbiturate anaesthetized spinal cats and remained in situ during either electrical stimulation of myelinated and unmyelinated fibres of the ipsilateral tibial nerve or pinching of digits of the ipsilateral hind paw. They were then incubated for 24 hours in ^{125}I Bolton-Hunter SP, washed and placed on x-ray film. The resultant autoradiographs were quantitatively analysed with an image analysis system and plots of optical density of microprobes were stored on a hard disk. Deficits in binding of the labelled peptide were equated with bound endogenous peptide. Both forms of peripheral stimulus significantly increased levels of irSP predominantly in the region of the substantia gelatinosa. The endopeptidase inhibitor kelatorphan was microinjected as a 10^{-3}M solution; either as a single injection of 0.5 μl in the region of the substantia gelatinosa or 4 injections, each of 0.1 μl, in the upper and lower dorsal horn. Microprobes inserted after kelatorphan injection and during peripheral stimulation showed wide spread presence of irSP extending throughout the dorsal columns and down to the ventral horn. These results suggest that inactivating enzymes can determine the sites accessed by a synaptically released neuropeptide and this may be an important differentiating factor with co-released compounds. Since calcitonin gene related peptide has been shown to inhibit SP degradation this control may have physiological significance.

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228P CHARACTERIZATION OF NEUROKININ RECEPTORS IN THE RAT NUCLEUS ACCUMBENS

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In the rat nucleus accumbens (NAS), substance P (SP) and neurokinin A (NKA)-containing terminals are in close proximity to tyrosine hydroxylase containing terminals (Pickel *et al.*, 1988). Microinfusion of SP into the NAS increases both the local release of dopamine (DA) and locomotor activity (LMA) (Kalivas and Miller, 1984) whereas intra-NAS infusion of NK antibodies has the opposite effect (Elliott *et al.*, 1986). In the present study we have attempted to characterize the NK receptors mediating this increase in LMA. The selective agonists used were GR73632 (NK-1), GR64349 (NK-2) (Hagan *et al.*, 1991) and senktide (NK-3).

Male Lister hooded rats (220-270 g; Glaxo, Ware) were chronically implanted with bilateral cannulae aimed at the NAS. One week following surgery the animals were microinfused bilaterally with 1.0 μl of drug or vehicle over a 1.0 min period. Subsequently, LMA was recorded over a 1.0 h test session in photocell cages. Histological verification of cannulae placement was determined upon termination of the study and only results from animals with correct placements were included in the data analysis (ANOVA and post-hoc Dunnett's test).

The NK-1 agonist, GR73632, produced a dose-dependent (0.1 - 10 nmol) increase in LMA which was significant ($p<0.05$) at doses above 0.3 nmol and maximal at 3.0 nmol. Photocell beam counts h^{-1}:- vehicle: 680 ± 56 (n=16); GR73632 at 0.03 nmol: 809 ± 73 (n=9); 0.3 nmol: 981 ± 206 (n=9); 1.0 nmol: 1077 ± 136 (n=11); 3.0 nmol: 1324 ± 110 (n=15); 10nmol: 1309 ± 147 (n=8). Intra-NAS infusion of the selective NK-2 and NK-3 agonists (at doses up to 3.0 nmol) did not significantly alter LMA (101% and 107% of control levels respectively) or induce other behaviours. The LMA induced by intra-NAS GR73632 (1.0 and 3.0 nmol) was shown to be mediated via a DA mechanism as haloperidol (0.1 mg/kg; s.c.) abolished the motor response of both doses. Haloperidol itself had no effect on LMA.

In conclusion, we have shown that the locomotor effects of the endogenous NKs, within the rat NAS, are probably mediated via NK-1 receptors which is supported by recent work by McLean et al (1991) who have shown a high density of NK-1 receptor binding sites in this area.

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229P ^{125}I-NEUROPEPTIDE-γ AND ^{125}I-IODOHISTIDYL-NEUROKININ A LABEL MULTIPLE TACHYKININ RECEPTORS IN RAT BRAIN

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Neuropeptide-γ (NP-γ) is an N-terminally-extended form of neurokinin A (NKA) derived from γ-preprotachykinin. In this study, the presence of central NK_2 receptor sites has been investigated using ^{125}I-NP-γ and ^{125}I-iodohistidyl-NKA (^{125}I-NKA) as radioligands. The pharmacological profile of the binding of each ligand to rat hippocampal membranes and brain sections has been determined using tachykinin receptor-selective agonists or antagonists as selective displacers at NK_1, NK_2 and NK_3 receptor sites.

Methods used for ^{125}I-NP-γ and ^{125}I-NKA receptor binding and autoradiography were as previously described (Dam et al., 1990). Hippocampal membranes (200μg protein) were incubated with either ^{125}I-NP-γ (50pM) or ^{125}I-NKA (100pM) in the presence or absence of increasing concentrations (1pM-30μM) of the NK_1-selective agonist, [Sar^9,Met(O$_2$)^{11}]SP (Sar^9), the NK_2-selective antagonist, L-659,877 or the NK_3-selective agonist, senktide. Non-specific binding was determined in the presence of 1μM NP-γ or 1μM NKA, respectively. For receptor autoradiography, 20μm coronal rat brain sections were incubated with ^{125}I-NP-γ (25pM) or ^{125}I-NKA (50pM) in the presence or absence of 1nM Sar^9, 30nM L-659,877 or 100nM senktide. Non-specific binding was determined as above.

Table 1. Inhibition of ^{125}I-NP-γ and ^{125}I-NKA binding to hippocampal membranes by NK_1, NK_2 and NK_3-selective displacers. Data are mean pIC_{50}s ± sem and mean % inhibition ± sem for high affinity displacement (n = 3).

	^{125}I-NP-γ		^{125}I-NKA	
Sar^9	10.34 ± 0.06	71.6 ± 1.7%	10.32 ± 0.23	75.5 ± 2.9%
L-659,877	9.34 ± 0.25	18.7 ± 0.9%	8.35 ± 0.28	18.4 ± 3.1%
Senktide	-	-	8.55 ± 0.62	25.0 ± 6.5%

Both ^{125}I-NP-γ and ^{125}I-NKA binding were displaced biphasically by Sar^9 and L-659,877 (Table 1). In addition, ^{125}I-NKA binding could be displaced by nM concentrations of senktide. Low affinity labelling was displaced by micromolar concentrations of displacers. Consistent with this, binding of ^{125}I-NP-γ to rat brain sections (determined autoradiographically) was displaced from most brain regions by Sar^9 (except thalamus and CA1, CA2 and CA3 of hippocampus) while L-659,877 displaced only from thalamus and hippocampus (but not dentate gyrus). Senktide was ineffective at 100 nM. In contrast, ^{125}I-NKA binding was displaced by all three displacers (at varying degrees and from different brain structures).

These results clearly indicate that ^{125}I-NP-γ and ^{125}I-NKA label multiple tachykinin receptors in rat brain. Approximately 18% of the sites labelled in hippocampus are sensitive to the NK_2 antagonist L-659,877 and most likely represent NK_2 receptors.

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230P THE NON-PEPTIDE TACHYKININ NK-1 RECEPTOR ANTAGONIST, CP-96,345, MAY DISCRIMINATE SPECIES VARIANTS IN NK-1 RECEPTORS

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The tachykinin NK-1 receptor antagonist, CP-96,345 [(2S,3S)-*cis*-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine] inhibits the binding of [^{3}H]-substance P (SP) to brain membranes but appears less potent in rat than in bovine tissue (Snider et al., 1991). This could indicate the existence of subtypes of the NK-1 receptor. To investigate this, we have measured the ability of racemic CP-96,345 to displace [^{3}H]-SP binding from brain cortex membranes prepared from six different species, including man, and have confirmed affinity estimates using functional assays *in vitro*.

Cerebral cortical membranes (8-15mg wet weight/assay tube) were incubated with [^{3}H]-SP (0.5-0.7nM) for 40 min at 22° C. The reaction was terminated by rapid filtration and bound radioactivity determined. CP-96,345 potently inhibited binding of [^{3}H]-SP to rabbit, guinea-pig, human and bovine cerebral cortices with similar nanomolar potencies (Table 1). In contrast, CP-96,345 was much less potent in rat or mouse tissue (Table 1). In comparison, the NK-1 agonist physalaemin was equipotent in all species (Table 1). Saturation analysis indicated that [^{3}H]-SP bound to rabbit and rat cerebral cortices with K_D values of 0.20 ± 0.01nM and 0.17 ± 0.01nM respectively. Using these determinations, pKi values for CP-96,345 in rabbit and rat cortex were calculated to be 9.30 ± 0.08 and 7.47 ± 0.07 respectively. In functional assays, CP-96,345 behaved as a reversible competitive antagonist of substance P methylester- (SPOMe)-induced relaxation of phenylephrine-contracted rabbit thoracic aorta (Hagan et al., 1990) or of SPOMe-induced depolarization of neonatal rat hemisected spinal cord (Ireland et al., 1988). Estimates of the equilibrium dissociation constant (pK_B) were 8.79 ± 0.07 (n=16) and 7.13 ± 0.10 (n=15), respectively. These are in good agreement with the estimates of binding affinity obtained against [^{3}H]-SP in rabbit and rat cerebral cortex.

Table 1

	Inhibition of binding of [^{3}H]-SP (pIC_{50})					
	Rabbit	Guinea-pig	Human	Bovine	Rat	Mouse
CP-96,345	8.62 ± 0.06	8.50 ± 0.08	8.46	8.86 ± 0.12	6.77 ± 0.08	6.92 ± 0.08
Physalaemin	8.13 ± 0.05	7.92 ± 0.10	n.d.	8.10 ± 0.01	8.12 ± 0.08	8.25 ± 0.09

Results are mean ± s.e. mean of 3-9 experiments, except human (n=1). Slopes of displacement curves were not significantly different to unity.

The present results support the contention that the affinity of CP-96,345 for NK-1 receptors is species-dependent, perhaps indicating that NK-1 receptors can be resolved into two groups, those in rabbit, guinea-pig, human and cow being distinct from those in rat and mouse.

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231P DISTRIBUTION OF CCK RECEPTOR SUBTYPES IN RAT AND CHICK BRAIN

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Recently use has been made of highly selective non-peptide CCK antagonists to map CCK receptor subtypes in rodent and monkey brain (Hill and Woodruff 1990).In all species studied CCK_B was the predominant receptor subtype with CCK_A receptors occurring at high density in discrete areas of the brain such as the interpeduncular nucleus, ventral tegmental area (VTA) and nucleus tractus solitarius (NTS). The present study extends these findings to examine the CCK receptor subtypes in the avian brain.

The distribution of receptors in chick (21 day old) and rat brain was compared autoradiographically in 20µm sections taken from frozen brains and thaw mounted onto gelatin-coated slides. Binding was at room temperature for 2 hours in the presence of 50pM [^{125}I]-Bolton-Hunter-CCK. Total displaceable binding was defined by the addition of CCK-8 (1µM), binding to CCK_A receptors by the addition of 10nM devazepide and to CCK_B receptors by 100nM CI-988. Similar conditions were used for ligand binding studies with washed membranes prepared from chick forebrain and rat cortex.

Both rat and chick showed high densities of CCK displaceable binding. The rat had high densities of CCK_B receptors in the cortex and striatum and high densities of CCK_A in the VTA and NTS. This pattern of subtype distribution is as previously described (Hill and Woodruff, 1990). In contrast in the chick brain (125I)-BH CCK binding was high in cortex, corpus striatum and hypothalamic areas. This binding was 80% displaceable by the CCK_A antagonist devazepide, and no area with a high predominance of CCK_B receptors, as defined by CI-988, was found. The unexpectedly high proportion of CCK_A receptor subtypes in chick brain was confirmed by displacement studies with a second CCK_A antagonist, lorglumide. Studies of ligand binding to both rat and chick membranes confirmed that in rat cortex the CCK receptors are mainly of the CCK_B subtype, CI-988 potently displaced binding with an IC50 of 0.9 nM (range 0.8-1.5 n=3) and devazepide displaced with an IC50 of 200nM (range 100-300 nM n=3) which is consistent with the CCK_A antagonist displacing from the CCK_B subtype at this concentration. In contrast in chick forebrain membranes both CCK_A and CCK_B receptor subtypes were present CI988 displaced some 20-30% of the binding with an IC50 of 1.1 nM (0.7-1.5 nM n=3) and devazepide displaced 70-80% of the binding with an IC50 0.3nM (0.1-0.4 n=3).

The results show further interesting variations in the distribution of CCK receptor subtypes in CNS tissue. In avian brain both A and B are present in all areas with the CCK_A receptor subtype being the predominant species. This is in contrast with mammalian species where the CCK_B receptor subtype is the main CNS receptor.

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232P DIFFERENT EFFECTS OF CHOLECYSTOKININ (CCK) ON THE ELECTROENCEPHALOGRAM (EEG) OF UNANAESTHETISED RATS AND SHEEP

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Exogenous administration of CCK causes a reduction of food intake in a number of animal species (see Ebenezer et al., 1990). Furthermore, the observations in rats that systemically administered CCK produces behavioural and EEG changes that are normally seen after a meal, such as those associated with resting and sleep, have suggested a role for this peptide as a satiety factor. The aim of this study was to compare the effects of CCK at doses that have been shown to inhibit feeding on the EEG of rats and sheep. Female Wistar rats (n=6) and male Dalesbred sheep (n=3) were implanted under equithesin (rats) or halothane (sheep) anaesthesia with stainless steel EEG screw electrodes. The methods for chronic recordings of EEGs from unanaesthetised rats and sheep, and the subsequent off-line spectral analysis of the data have been described previously (Ebenezer, 1987; Ebenezer et al., 1991). EEGs were recorded from the rats prior to, and following i.p. administration of CCK (1, 4 and 8µg/kg), and from the sheep prior to, and following both i.v. (0.25, 0.5, 1 and 2µg/kg) and s.c. (0.5, 1 and 4µg/kg) injections of CCK. Physiological saline was administered in control experiments by the appropriate routes.

All doses of CCK produced synchronization of the EEG in the rats during the 1st 20 min after administration, which is consistent with a decrease in the level of arousal of the animals. This was characterized by significant increases ($P<0.01$) in spectral power in the 0 - 3.5Hz frequency band. Thus, e.g., the 4µg/kg dose increased the mean power by 55% in this frequency band during the 1st 10 min after administration. In contrast, none of the doses of CCK given i.v. or s.c. to sheep produced any effects on the EEG. As a positive control we demonstrated that sodium methohexitone (0.5mg/kg) produced an almost immediate synchronization of the EEG in these sheep.

The present results thus indicate (i) that there is a definite species difference in the EEG response of rats and sheep to systemic administration of CCK, and (ii) that EEG changes associated with a prandial resting state are not observed in sheep after CCK.

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233P EFFECT OF L-365,260 ON THE RELEASE OF CHOLECYSTOKININ-LIKE IMMUNOREACTIVITY FROM RAT NUCLEUS ACCUMBENS *IN VITRO*

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In a previous study (Gupta and Ceasar, 1991), we showed that blockade of Type-A cholecystokinin (CCK_A) receptors leads to an increase in the amount of cholecystokinin-like immunoreactivity (CCK-LI) released from the rostral nucleus accumbens (NAc) of the rat, following administration of veratrine. We have now investigated the effects of L-365,260, a selective antagonist of CCK_B receptors (Lotti and Chang, 1989), on the release of CCK-LI from superfused slices of the rostral and caudal NAc respectively. The CCK-LI, released in response to bolus doses of veratrine, was analysed by radioimmunoassay, using a C-terminal-specific antibody.

In the presence of L-365,260, an increase in the CCK-LI released from the rostral NAc during the 0-2 min and 2-4 min time intervals after the administration of submaximal doses of veratrine was observed (Table 1).

Table 1. Release of CCK-LI from the rostral NAc in response to veratrine (12.5μg).

	L-365,260 (10nM)	Control	L-365,260 (100nM)	Control
0-2 min	7.04 ± 1.27*	0.76 ± 0.52	3.57 ± 1.26*	0.55 ± 0.27
2-4 min	12.87 ± 2.80*	3.86 ± 1.26	8.04 ± 2.61*	2.83 ± 0.98

The data are the mean ± s.e. mean values (pg/min/mg protein) for 6 experiments. * $P<0.05$, Mann-Whitney U test.

In contrast, there was no significant alteration in the amounts of CCK-LI released from the caudal region of the NAc during either time period.

The increase in the evoked release of CCK-LI from the rostral NAc, following antagonism of CCK_B receptors, suggests a role for CCK_B receptors in modulating the release of CCK-like peptides from neurones of this region. However, the precise location of such receptors in relation to the CCK neurones is unclear at present.

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234P A GREASE-GAP STUDY OF THE EFFECTS OF HYPOXIA ON ELECTRICAL ACTIVITY OF HIPPOCAMPAL CA1 NEURONES

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We have used a quantitative electrophysiological method, the grease-gap technique for the hippocampal slice (Blake et al 1988), to examine the population response of CA1 neurones to periods of hypoxia (Rogers et al 1990). The effects of such treatment upon D.C. potential, agonist-induced depolarisations and electrically-evoked field EPSPs are described.

Hippocampal slices were prepared from adult female albino rats and were continously perfused with standard 1 mM Mg^{2+}, 10 mM D-glucose-containing aCSF at 28 °C bubbled with 95% O_2/5% CO_2. A potential difference was recorded between two wick electrodes placed either side of a grease barrier which separated the alveus from the remainder of the slice from which the CA3 region was sometimes removed. The application of the glutamate receptor agonists NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) to the perfusate (for 90 s) elicited dose-dependent depolarisations with a maximum response of approx 1 mV. Field EPSPs were recorded across the grease-barrier in response to electrical stimulation of the stratum radiatum. Hypoxic episodes were produced by substituting, for 5-40 min, the control aCSF with identical aCSF equiliberated with 95% N_2/5% CO_2.

The predominant (17/25 slices) sequence of D.C. potential changes in response to hypoxia were; 1) an early hyperpolarisation, 2) a depolarisation and 3) a characteristic hyperpolarisation on reoxygenation. The initial hyperpolarisation (within 2-3 min of 95% N_2) was coincident with a depression of the evoked EPSP. However, this early depression of synaptic transmission was not reflected by a decrease in postsynaptic sensitivity to NMDA and AMPA (3 and 4 slices, respectively). If the hypoxic episode was continued for > 4 min, the initial hyperpolarisation reversed into a depolarisation of which two types were observed. A slow, shallow depolarisation or a rapid, steep depolarisation. This latter depolarisation, if uninterrupted by a return to normoxia, was several mV in amplitude and was associated with incomplete or no recovery of the evoked EPSPs on reoxygenation. Paradoxically, during prolonged hypoxia (> 10 min) the evoked EPSPs displayed a transient partial recovery (4 slices) which faded as the hypoxic episode continued. This too was a precursor to partial or no recovery of the evoked EPSPs after normoxia had been re-established.

The application of agonists, selective for those receptors mediating transmission onto CA1 cells, during the initial stages of hypoxia suggests that the early depression of synaptic transmission is presynaptic in origin. The temporal contribution of pre and postsynaptic structures to the observed potential changes and transient recovery during hypoxia will be the subject of future studies.

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235P PURINE INHIBITION IS REDUCED DURING NMDA STIMULATION OF SINGLE PYRAMIDAL CELLS IN THE RAT HIPPOCAMPAL SLICE

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The neuromodulator adenosine acts in the central nervous system to depress synaptic transmission. This action, however, may be moderated by NMDA receptor activation. In a recent study (Bartrup and Stone 1990) we reported that NMDA receptor channel activation appears to reduce the inhibitory action of adenosine on evoked population potentials in the hippocampal slice. We have extended this study to look at single hippocampal pyramidal cells in the slice preparation.

Responses of cells to microiontophoretic application of drugs were recorded extracellularly. The micropipette was filled with N-methyl D,L-aspartate (NMA)(10mM), quisqualic acid (10mM), acetylcholine (100mM), adenosine 5,-monophosphate (AMP)(100mM) and 4-aminobutyric acid (GABA)(1M). Cell firing was recorded via a window discriminator and instantaneous ratemeter onto a Grass chart recorder.

Single cells were stimulated by continuous iontophoretic application of acetylcholine (10-50nA). A submaximal plateau of firing was attained after which 5 second pulses of AMP were ejected at regular 60 second intervals to give at least 3 comparable inhibitory responses. The AMP current was set to give a just maximal inhibition of cell firing and this current was maintained throughout the experiment. This process was repeated with quisqualic acid (1-10nA) and NMA (1-10nA) as the neuroexcitants with a 5 minute rest between each. Currents were adjusted to give a level of firing comparable to that induced by acetylcholine. The level of AMP inhibition was measured as the area of inhibition recorded on the ratemeter trace. AMP inhibition under acetylcholine stimulation was taken as a 100%. There was a small but significant decrease in the level of AMP inhibition during quisqualate stimulation, 79±9.8% of that seen with acetylcholine ($n=14$, $p<0.05$). Under NMA stimulation however, AMP inhibition was reduced to 29±4.5% ($n=14$, $p<0.001$). Inhibition could be restored to 100% if the AMP ejection current was increased approximately 3 fold. Experiments were also performed with GABA as the inhibitory agent, but the level of GABA inhibition was the same under all 3 neuroexcitants.

These results are consistent with our previous conclusion that the inhibitory properties of purines are impaired during activation of NMDA channels. The mechanism by which this may occur is unclear. Adenosine and NMDA receptors may act on a shared calcium channel competitively antagonizing each other as suggested by a recent report (Chernevskaya et al., 1991). An interaction between adenosine and NMDA may have important implications for NMDA receptors mediated toxicity during ischaemia and seizures as well as neuronal phenomena such as long term potentiation.

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236P PRESYNAPTIC INCREASES IN $[Ca^{2+}]_i$ INDUCED BY QUISQUALATE AND CARBACHOL ARE TEMPORALLY DIFFERENT AND ARE MEDIATED BY DISTINCT MECHANISMS

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Previous studies in this laboratory have shown that the glutamatergic receptor agonist quisqualate and muscarinic cholinergic receptor agonist carbachol increase intrasynaptosomal free calcium ($[Ca^{2+}]_i$) but that these effects are achieved by different mechanisms, i.e. carbachol increases $[Ca^{2+}]_i$ by facilitating Ca^{2+} entry through L-type Ca^{2+} channels via a 1,2-diacylglycerol (DAG)-protein kinase C (PKC)-dependent pathway while quisqualate mobilises Ca^{2+} from inositol 1,4,5-trisphosphate (IP_3)-sensitive stores (Adamson et al., 1990; Boess et al., 1990; Xiang et al., 1991).

The present study extended these issues by examining the temporal relationship between the effects of quisqualate and carbachol. Rat cortical synaptosomes were used and changes in $[Ca^{2+}]_i$ in Fura-2-loaded synaptosomes induced by the agonists were measured using an Aminco Bowman spectrofluorometer: the settings of which were fixed at 340 nm (excitation) and 500 nm (emission), respectively. Calculation of $[Ca^{2+}]_i$ were carried out as previously described (Xiang et al., 1991).

The data demonstrated that both quisqualate (10 µM) and carbachol (100 µM) increased $[Ca^{2+}]_i$ but that in each case, the increase was much slower than that induced by K^+-evoked depolarisation. In addition, the return to the basal $[Ca^{2+}]_i$ level was not complete for approximately 300 sec. Interestingly, the temporal profiles were substantially different. The increases in $[Ca^{2+}]_i$ induced by carbachol reached a maximum (750 nM) within 90 sec and declined relatively rapidly, i.e. by 50% within 150 sec ($n=4$). On the other hand, the increases induced by quisqualate were slower and did not reach a maximum (580 nM) until 210 sec: in addition, the profile was different in that a value close to the maximum persisted for approximately 100 sec ($n=5$).

As described above, the carbachol-mediated increase in $[Ca^{2+}]_i$ is due to opening of L-type Ca^{2+} channels in the plasma membrane and thus would be expected to be more rapid than the quisqualate-elicited release from IP_3-sensitive Ca^{2+} stores, which are likely to be located deeper within the nerve terminals. The temporal differences shown in the present study provide some evidence that the activation of these two presynaptic receptor types may be involved in the mediation of different cellular processes: the effects of carbachol may be related to the acute control of release of neurotransmitters whereas the effects of quisqualate may lead to the modulation of more long-term intracellular changes, e.g. those related to synaptic plasticity.

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237P REVERSIBLE HOMO- AND HETEROSYNAPTIC DEPRESSION OF SYNAPTIC TRANSMISSION INDUCED BY THE ACTIVATION OF NMDA RECEPTORS IN THE CA1 REGION OF THE RAT HIPPOCAMPUS

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The activation of N-methyl-D-aspartate (NMDA) receptors has been implicated in models of plasticity, such as long-term potentiation (LTP), as well as in various pathological states, such as epilepsy and cell death (Watkins and Collingridge, 1989). We are currently investigating the involvement of NMDA receptors in an activity-dependent depression of synaptic transmission in the CA1 region of the hippocampus.

Hippocampal slices were perfused with warmed (30°C) Krebs solution bubbled with O_2/CO_2 (95%/5%) and comprising (mM) NaCl 124, NaH_2PO_4 1.25, $NaHCO_3$ 26, KCl 3, $CaCl_2$ 2, $MgSO_4$ 1 and D-glucose 10. Field EPSPs were recorded from the stratum radiatum. The Schaffer-collateral commissural pathway was stimulated via two electrodes placed either side of the recording electrode; each pathway was stimulated alternately (every 60s) in order to evoke synaptic responses of 1-2 mV in amplitude. Experiments were conducted in the presence of the $GABA_A$ antagonist picrotoxin (50 μM) to unveil an NMDA receptor-mediated component, in addition to the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated component of synaptic transmission.

Tetanic stimulation (100 Hz, 1sec, test stimulus intensity) resulted in homosynaptic LTP of both components of the synaptic response (n=6). However, a tetanus (100 Hz, 1 sec) delivered at twice the test stimulus intensity invariably resulted in homo- and heterosynaptic depression of both synaptic components (n=6) which recovered within 30 min. The induction of depression, as with LTP, was reversibly blocked by bath application of the NMDA receptor antagonist (AP5; 20 μM, n=3). The depression of synaptic activity was associated with a large (10-25 mV), transient (1-2 min) extracellular dc shift which was not blocked by the Ca^{2+} channel blockers nitrendipine (n=4), nimodipine (n=3) or verapamil (n=2; all at 20 μM), but was reversibly blocked by AP5 (n=3; 20 μM).

These results implicate the transient activation of NMDA receptors in the induction of an activity-dependent homo- and heterosynaptic depression of synaptic transmission in the CA1 region of the rat hippocampus.

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238P EVIDENCE THAT SEIZURES AND NEURONAL CELL DEATH PRODUCED BY INTRAHIPPOCAMPAL TETANUS TOXIN IN RATS CAN BE REDUCED BY CGP 37849 AND CARBAMAZEPINE

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Intrahippocampal injection of tetanus toxin (TT) in rats produces motor and electrographic (ECoG) seizures and delayed brain damage. These effects may stem from TT-induced inhibition of GABA release leading to a predominance of glutamic acid-mediated excitation. In fact, systemic CGP 37849, a competitive N-methyl-D-aspartate receptor antagonist (Schmutz *et al.*, 1990), can prevent neuronal damage produced by TT (Bagetta *et al.*, 1991). To further support our hypothesis we have now studied the ECoG profile of TT-injected rats systemically treated with CGP 37849. In addition, possible neuroprotection afforded by carbamazepine, an antiepileptic drug able to prevent or to reduce motor and ECoG epileptic discharges induced by TT (Hawkins and Mellanby, 1986), was also studied.

Male Wistar rats (250-280g) were monolaterally injected with a single dose (1000 MLDs) of TT as previously described (Bagetta *et al.*, 1991). Four stainless steel screw electrodes were chronically placed on to each fronto-parietal cortex surface for ECoG recordings. CGP 37849 (10mg/kg i.p.) was administered once daily for 10 days or carbamazepine (30mg/kg/day) was given orally in the drinking water for the same period. Histological examination of the hippocampi was performed as previously described (Bagetta *et al.*, 1991). In control rats (water vehicle n=10), TT alone produced motor fits and ECoG spikes (single or bursts) in all animals with a latency ranging from 2 to 4 days; bursts or spikes lasted 30sec -2min. Only 1 rat survived at day 10. In rats receiving CGP 37849 motor and ECoG discharges were observed in only 1 out of 7 rats and these commenced 5 days after TT injection. At 10 days 4 out of 7 rats survived. TT produced motor seizures in 3 out of 10 rats treated with carbamazepine and 6 rats survived at day 10. In these animals, histological examination of coronal brain sections showed no hippocampal damage.

In conclusion, the results support our original contention that unopposed excitatory amino acid receptor activation may be responsible for the convulsant and neurodegenerative effects of TT. However, the mechanism of the apparent protection afforded by carbamazepine remains to be resolved. We thank CIBA-Geigy, Basel for CGP 37849 and carbamazepine.

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239P [^{3}H]DIZOCILPINE AND [^{3}H]CPP BINDING IN THE DEVELOPING RAT BRAIN

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The N-methyl-D-aspartate (NMDA) receptor has been implicated in both physiological and pathological events in the developing central nervous system (CNS) (Brewer and Cotman, 1989; Olney et al, 1989). Radioligand binding using NMDA receptor antagonists has been used to follow the ontogeny of the NMDA receptor neurotransmitter site and the associated ion channel from postnatal day 0 (PND0) to PND90.

Synaptosomal membranes were prepared from pooled rat cortex and hippocampus. [^{3}H]3-((±)-2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid ([^{3}H]CPP 10nM) binding was measured using a microcentrifugation assay and [^{3}H]dizocilpine (1nM) using a filtration assay (Olverman et al, 1986; Wong et al, 1986). The findings are presented in Table 1. Saturation analysis using unlabelled ligand showed that for both CPP and dizocilpine, the K_d values remained constant throughout development.

Comparable patterns of binding during development were seen with both ligands suggesting parallel ontogenic profiles. Thus binding of both ligands increased sharply from PND0 reaching a plateau at PND13-16. The increases in binding of the two ligands during postnatal development were due to an increase in receptor density and not to a change in receptor affinity. The presence of 10μM glycine alone or in combination with 10μM L-glutamate increased [^{3}H]dizocilpine binding throughout development, by increasing its affinity. This indicates that the NMDA glycine site must also be present from PND0 and that all three sites may be functionally coupled from an early stage in development.

Table 1 Specific binding of 10nM [^{3}H]CPP and 1nM [^{3}H]dizocilpine to synaptosomal membranes (fmol/mg protein)

Age range	CPP	dizocilpine	dizocilpine*
0-4 (days)	67.7 ± 10.7(4)	49.5 ± 11.2(8)	302.1 ± 23.8(9)
5-8	63.8 ± 23.8(3)	223.1 ± 54.3(5)	813.3 ± 138.5(5)
9-12	124.8 ± 10.6(7)	296.0 ± 107.8(4)	1165.2 ± 286.9(4)
13-16	138.4 ± 18.1(8)	645.0 ± 54.9(4)	1560.9 ± 75.5(4)
17-20	196.3 ± 10.0(4)	593.9 ± 205.5(4)	1949.9 ± 560.0(4)
21-24	343.1 ± 19.9(5)	624.5 ± 156.4(3)	1634.5 ± 215.6(3)
25-28	nm	572.0 ± 216.2(3)	1779.1 ± 455.5(3)
90	305.7 ± 17.2(23)	331.6 ± 50.6(17)	1852.9 ± 179.8(17)

* in the presence of 10μM L-glutamate and 10μM glycine. Values represent mean ± s.e. mean. n.m. not measured. Figures in brackets refer to number of observations.

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240P THE NEUROPROTECTIVE AGENT RILUZOLE INHIBITS RELEASE OF L-GLUTAMATE AND L-ASPARTATE FROM SLICES OF HIPPOCAMPAL AREA CA1

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Riluzole possesses anticonvulsant and neuroprotective activity in animal models. It is believed to exert its effects by reducing glutamate release (Malgouris et al., 1989). This study examined the effects of riluzole on the release of glutamate and aspartate from the Schaffer collateral-commissural-ipsilateral associational (SCCIA) pathway in area CA1 of the rat hippocampus. Transmitter release was evoked from superfused slices of the CA1 area (excluding stratum lacunosum-moleculare) by exposing the slices to 1-min pulses of 50 mM K^+ (see Burke & Nadler, 1988). Under these conditions nearly all the release of L-glutamate, L-aspartate and GABA is Ca^{2+}-dependent and it originates predominatly from SCCIA pathway.

Riluzole (10-100 μM), in the absence or presence of 1.2 mM Mg^{2+} significantly inhibited the release of both L-glutamate and L-aspartate in a concentration-dependent manner. It depressed the release of these amino acids about equally and by as much as 40%. Only the highest concentration of riluzole (100 μM) significantly inhibited GABA release. TTX (0.1 μM) reduced the K^+-evoked release of L-glutamate and L-aspartate by about 40%, suggesting that a portion of the release depended on the generation of action potentials as the K^+-concentration within the tissue was rising. The action of riluzole (30 μM) was not confined to the TTX-sensitive component of the release since a further significant reduction in the evoked L-aspartate and L-glutamate release was observed.

Therefore the anticonvulsant and neuroprotective properties of riluzole may be at least partly explained by its ability to inhibit L-glutamate/L-aspartate release from synaptic terminals. In general, the actions of riluzole resembled those of NMDA receptor antagonists and inhibitors of arachidonic acid metabolism that act upon the lipoxygenase pathways (Martin et al., 1991; Duncan et al., 1990). One possibility is that riluzole either inhibits the breakdown of arachidonic acid by lipoxygenases or interferes with the up regulation of L-glutamate/L-aspartate release by lipoxygenase products. (Supported by NIH grant NS16064).

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241P DIFFERENTIAL MODULATION OF [^{3}H]GLYCINE AND [^{3}H]L-689,560 BINDING TO THE GLYCINE SITE ON THE NMDA RECEPTOR BY GLUTAMATE SITE ANTAGONISTS

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Ligands for the glutamate recognition site of the N-methyl-D-aspartate (NMDA) receptor modulate binding at the associated glycine recognition site (Monahan et al, 1990). Antagonists containing 5 carbon atoms (C-5) are reported to reduce [^{3}H]glycine binding whereas those containing 7 carbon atoms (C-7) have significantly less effect (Monahan et al, 1990). We have investigated the effects of C-5 antagonists (D-2-amino-5-phosphonopentanoate (D-AP5) and CGP37849), C-7 antagonists (3-[(±)-2-carboxypiperazin-4-yl]propyl-1-phosphonate (CPP) and D-2-amino-7-phosphonoheptanoate (D-AP7)), NMDA and L-glutamate on the binding of the glycine antagonist [^{3}H]L-689,560 (Grimwood et al, this meeting). P_2 membranes were prepared from rat cortex and hippocampus and used to determine [^{3}H]L-689,560 binding (Grimwood et al, this meeting) and [^{3}H]glycine binding (Foster and Kemp, 1989).

Table.

	[^{3}H]Glycine		[^{3}H]L-689,560	
	EC_{50}(μM)	% Max	EC_{50}(μM)	% Max
D-AP5 (C-5)	0.39(0.29,0.51)	52±0.1↓	0.062(0.034,0.113)	36±3.9↓
CGP37849 (C-5)	0.021(0.010,0.046)	59±6.5↓	0.013(0.009,0.018)	30±2.7↓
D-AP7 (C-7)	NE	NE	1.29(0.91,1.98)	50±4.8↓
CPP (C-7)	NE	NE	0.292(0.252,0.338)	54±1.2↓
NMDA	NE	NE	1.61(1.29,2.00)	30±4.3↑
L-glutamate	NE	NE	NE	NE

EC_{50} values (geometric mean (-SEM,+SEM)) and % maximal effects (arithmetic mean ± SEM) are for 4 experiments. ↑enhancement, ↓inhibition of binding. Incubation time for [^{3}H]L-689,560 binding was 45 min. NE = no effect.

The C-5 and C-7 antagonists had differential effects in the two binding assays. The C-7 antagonists inhibited [^{3}H]L-689,560 binding exclusively. The C-5 antagonists inhibited [^{3}H]glycine binding with EC_{50} values similar to their affinities for the glutamate recognition site, whereas at lower concentrations they inhibited [^{3}H]L-689,560 binding to a lower maximal extent (Table). NMDA enhanced [^{3}H]L-689,560 binding with an EC_{50} value similar to its affinity for the glutamate recognition site. These studies support the suggestion that when C-5 and C-7 antagonists bind to the glutamate recognition site of the NMDA receptor they induce different conformational states as reflected by changes in the affinity of glycine site agonists and antagonists.

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242P LIGAND BINDING TO THE N-METHYL-D-ASPARTATE RECEPTOR IN BRAINS FROM CONTROLS AND ALZHEIMER'S DISEASE PATIENTS

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Cortical pyramidal neurones use excitatory amino acids (EAAs) as transmitters, and contain receptors for EAAs. In Alzheimer's disease (AD), cortical pyramidal neurones are particularly susceptible to degeneration, thus implicating changes in EAAs and their receptors in the disease process. In the present study we have examined the binding of two ligands to the N-methyl-D-aspartate subtype of EAA receptor in brains from controls and patients with AD.

Autopsy samples of frontal (BA9/10) and temporal (BA 21/22) cortex were obtained from controls (n=8, mean age 79 ± 3y, post mortem delay, 26 ± 6h) and pathologically verified AD patients(n=8, 80 ± 3y, 24 ± 5h), and "total membrane" or "synaptosomal membrane" fractions prepared (Proctor et al 1991). NMDA receptors were labelled with either [^{3}H]-dizocilpine (1-40nM) or [^{3}H]-thienylcyclohexlpiperidine ([^{3}H]-TCP) (1-40nM) as described by Steele et al (1991), and maximum binding determined by Scatchard analysis. All assays were performed in the presence of maximally stimulating concentrations of glutamate (100μM) and glycine (1000μM) and non-specific binding determined with dizocilpine (10μM).

Table 1 [^{3}H]-Dizocilpine and [^{3}H]-TCP binding to frontal cortex of control and Alzheimer's disease patients

	Total membranes		Synaptosomal membranes	
	[^{3}H]-dizocilpine	[^{3}H]-TCP	[^{3}H]-dizocilpine	[^{3}H]-TCP
Control	1222 ± 161 (5)	897 ± 119 (5)	3317 ± 173 (8)	2534 ± 162 (8)
AD	1163 ± 157 (5)	827 ± 119 (5)	*2773 ± 180 (8)	2347 ± 246 (8)

Values are Bmax (fmol mgprotein^{-1}) mean ± s.e.mean (n) *$p<0.05$.

[^{3}H]-dizocilpine binding was significantly ($p<0.05$) reduced in the synaptosomal fraction of AD frontal cortex (Table 1). We have shown that [^{3}H]-dizocilpine binding is more sensitive to glycine stimulation than [^{3}H]-TCP binding (Steele et al 1991). The present finding may thus be related to the reduction in glycine modulation of the NMDA receptor in AD (Proctor et al 1989).

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243P THE BINDING OF [^{3}H]L-689,560, AN ANTAGONIST OF THE GLYCINE SITE ON THE NMDA RECEPTOR, TO MEMBRANES FROM POST-MORTEM HUMAN CEREBRAL CORTEX

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We have recently described the binding characteristics of [^{3}H]L-689,560, a high-affinity antagonist for the glycine site on the NMDA receptor (Leeson *et al,* 1991), to rat brain membranes (Grimwood et al - this meeting). The properties of [^{3}H]L-689,560 binding to rat brain membranes (K_D = 2 nM; 95% specific binding) suggested that it would be an ideal tool to study the glycine site in samples of human post-mortem brain. The membrane preparation was identical to that used to obtain rat P_2 membranes (Foster and Kemp, 1989) and employed human post-mortem cerebral cortex from 3 neurologically-normal subjects of 49, 55 and 59 years. Brains were frozen within 15 hours post mortem. [^{3}H]L-689,560 (0.1-100 nM) was incubated with 150 µg of membrane protein at 4°C for 120 min in 50mM Tris-acetate buffer (pH 7.0) in a final volume of 0.5ml. Non-specific binding was determined with 1mM glycine and bound radioactivity was separated by filtration through Whatman GF/B filters.

Saturation analysis of [^{3}H]L-689,560 binding (n=3) gave a B_{max} value of 0.954±0.015 pmol/mg protein (arithmetic mean±SEM) and a K_D value of 3.29 (3.01,3.60)nM (geometric mean(-SEM,+SEM)). Compared to values in the rat (Grimwood *et al,* this meeting), the K_D value is similar, but the B_{max} is approx. 30% in the human post-mortem tissue. The pharmacological specificity of the [^{3}H]L-689,560 binding site in human tissue was virtually identical to that observed in the rat. IC_{50} values (µM; geometric mean(-SEM,+SEM);n=3) for glycine site ligands were as follows: glycine: 0.53(0.28,1.02); D-serine 0.47(0.33,0.68); L-serine 44.4(29.6,66.8); R(+)3-amino-1-hydroxypyrrolid-2-one ((+)HA-966): 9.42(6.68,14.7); (-)HA-966: 851(696,1040); 7-chlorokynurenic acid: 0.273(0.168,0.442); L-689,560: 0.002(0.001,0.004). Comparison of these values with those obtained in the rat gave a 1:1 correlation with a correlation coefficient of 0.99. The glutamate site antagonists D-2-amino-5-phosphonopentanoate (D-AP5) and 3-[(-)-2-carboxypiperazin-4-yl]propyl-1-phosphonate (D-CPP) gave partial inhibition of [^{3}H]L-689,560 binding consistent with the allosteric effects observed in the rat (Grimwood, Wilde and Foster, this meeting).

These results indicate that the [^{3}H]L-689,560 binding site in human post-mortem cerebral cortex has properties which are virtually identical to those observed in the rat, suggesting equivalence between the glycine binding site on the NMDA receptor in the two species.

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244P THE EFFECT OF BTS 54 505 ON NMDA-EVOKED RESPONSES IN THE RAT DORSOLATERAL GENICULATE NUCLEUS AND HIPPOCAMPUS

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Tricyclic antidepressants have recently been shown to produce a voltage-dependent open channel block similar to the dissociative anaesthetics ketamine and MK-801 (Sernagor *et al,* 1989), and have also been shown to block NMDA-induced lethality in mice (Leander, 1989). The aim of this study was to determine the effect of BTS 54 505, the primary amine metabolite of the non-tricyclic putative antidepressant sibutramine HCl (Luscombe *et al,* 1989), on NMDA-evoked responses in the hippocampus and the dorsolateral geniculate nucleus (dLGN) in the rat.

Male Lister Hooded rats (200-250g) were anaesthetised with urethane (1.3-1.5 $g.kg^{-1}$, i.p.) and seven-barreled micropipettes were used to record extracellular neuronal discharge activity. The effect of ionophoresed BTS 54 505 (10mM; 0-40nA) on NMDA-evoked responses (10mM; 15s ejections (20nA) repeated at 120s intervals) was evaluated from integrated firing rate histograms. Ionophoresis of BTS 54 505 in the hippocampus and dLGN resulted in a current (dose)-dependent, reversible inhibition of the NMDA-evoked response in all cells studied (mean ED_{50}: hippocampus 21 ± 9nA, n=8 cells; dLGN 17 ± 7nA, n=8 cells); BTS 54 505 had no effect on spike waveform.

The current-dependent inhibition of the NMDA-evoked responses observed in this study is in agreement with results obtained using the whole-cell patch clamp technique (Sernagor *et al,* 1989, White *et al,* 1990), where desmethylimipramine produced a concentration-dependent decrease in the amplitude of the NMDA-activated current via open channel blockade. The inhibition of the NMDA-evoked response by the monoamine uptake inhibitor BTS 54 505 does not appear to be due to noradrenaline uptake inhibition since this latter action would cause a noradrenaline-evoked excitation in the dLGN (Rogawski and Aghajanian, 1980). Thus, elevation of endogenous monoamine levels appears to contribute little to the attenuation of the NMDA-evoked responses by ionophoresed BTS 54 505.

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245P A COMPETITIVE ANTAGONIST AT NMDA RECEPTORS, AT LOW DOSES, PROTECTS AGAINST THE BARBITAL WITHDRAWAL SYNDROME, BUT CROSS-TOLERANCE DOES NOT OCCUR

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The barbiturate withdrawal syndrome consists in humans of tremor, hyperreactivity and potentially fatal convulsions. This can be modelled in animal studies by ratings of tremor and convulsions. Recent reports showed that barbiturates, acutely, have a blocking actions at excitatory amino acid receptors (Sawada and Yamamoto, 1985). Changes in these systems may be involved in tolerance and physical dependence on barbiturates. We have therefore studied interactions with the competitive NMDA (N-methyl-d-aspartate) antagonist, CGP39551 (Pozza et al., 1990).

Male mice, TO strain, 20 - 25g, were given barbital in powdered food for 7 days (mean intake: 400 mg/kg/24h on day 1 rising to 750 mg/kg/24h on day 7). Controls received the same amount of food without drug. Withdrawal was measured by ratings of the convulsive behaviour on handling (Littleton et al., 1990). Groups of 10 barbital-treated mice were given CGP39551 2.5, 5 or 10 mg/kg, or saline vehicle, 19.5h after withdrawal from barbital. Ratings of behaviour were made hourly from 20h to 28h into withdrawal. The animals were coded for drug treatment. The first sets of behavioural ratings were as follows (median ± interquartile range):

Time	Controls	Vehicle	2.5 mg/kg	5 mg/kg	10 mg.kg
20h	0 (0-1)	2.5 (1-4)	1 (1-2.5)	0 (0-1)	0 (0-1)
21h	0 (0-1)	2.5 (2-3)	1 (0.5-2)	1 (0-1)	0 (0-1)
22h	0 (0-1)	2 (1-3)	1 (0.5-1.5)	0 (0-1)	0 (0-1)

Nonparametric analysis of variance on all the data: the effects of all doses of CGP39551 were significant ($P < 0.01$)

The barbital treatment did not alter the sedative actions of CGP39551, measured 24h after withdrawal. Once daily injections of CGP39551, 20 mg/kg, for 7 days, that caused tolerance to its sedative properties, did not alter the ataxic actions of pentobarbitone. In naive mice, CGP39551, at 10 mg/kg, prevented seizures due to NMDA, but not those to the GABA (γ-aminobutyric acid) antagonist, bicuculline, suggesting selectivity for events initiated at the NMDA site, compared with those initiated by GABA inhibition. At 2.5 mg/kg, CGP39551 was not sedative in naive mice.

These results suggest either that different mechanisms may underlie barbiturate tolerance and physical dependence, or that the anticonvulsant action of CGP39551 does not indicate that convulsive activity during withdrawal originates at NMDA sites. As the chronic barbital treatment did not alter the convulsive effects of NMDA (250 mg/kg NMDA, i.p., gave 5/10 seizures in controls and 5/10 seizures in barbital treated mice, 24h into barbital withdrawal), the latter explanation appears more likely.

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246P CHRONIC BARBITAL TREATMENT INCREASES MK801 BINDING

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Barbiturate dependence has been suggested to be due to decreased GABA inhibition, but this does not appear to be the whole explanation. Recent reports shpwed that barbiturates, acutely, have a blocking actions at excitatory amino acid receptors (Sawada and Yamamoto, 1985) and we have shown that the competitive NMDA antagonist CGP39551, at low doses, prevents the barbiturate withdrawal syndrome (Rabbani et al., 1991). We have measured receptor binding using [^{3}H]-MK-801 (disocilpine, a noncompetitive antagonist at NMDA sites) after chronic barbiturate treatment, compared with that after chronic treatment with a benzodiazepine agonist or the competitive NMDA antagonist, CGP39551.

Male mice, TO strain, 20 - 25g, were used throughout. Barbital was given in powdered food for 7 days (mean intake: 420 mg/kg/24h on day 1 rising to 700 mg/kg/24h on day 7). Controls received the same amount of food without drug. Flurazepam 40 mg/kg or saline vehicle was injected i.p. once daily for 7 days; CGP39551 or saline vehicle was given i.p. at 20 mg/kg once daily for 6 days. Dissection of cerebral cortices was carried out 24h after the removal of barbital from the food and 24h after the last injections of flurazepam or CGP39551. All the chronic treatments caused tolerance at these time intervals; the barbital and flurazepam treatment caused signs of withdrawal hyperexcitability, but no such signs were seen after CGP39551 treatment. Tissues were homogenised in Tris buffer and [^{3}H]-MK-801 binding measured using cold thienylcyclohexylpiperidine (TCP, 10 μM) to define nonspecific binding. Incubation was terminated after 45 min by rapid filtration through Whatman GF/B filters. The results were calculated by Scatchard analysis.

The barbital treatment caused a 32% increase in receptor density with no change in Kd. No significant changes were seen after the flurazepam treatment or the CGP39551. Results as follows: (n = 6 throughout)

Treatment	Bmax (pmol/mg protein)	Kd (nM)	Treatment	Bmax (pmol/mg protein)	Kd (nM)
Controls	6.35 ± 0.66	4.28 ± 0.41	Barbital	8.39* ± 0.96	5.17 ± 0.33
Vehicle	5.89 ± 0.21	5.5 ± 0.47	Flurazepam	5.83 ± 0.21	6.58 ± 0.85
Vehicle	6.55 ± 0.28	3.64 ± 0.24	CGP39551	6.84 ± 0.2	3.47 ± 0.1

*$P < 0.01$, by two-way analysis of variance

The results indicate that chronic barbital treatment caused upregulation of the [^{3}H]-MK-801 site, with no change in affinity, even though acute application of barbiturates does not appear to block NMDA activity. This change is intriguing in view of the depression of long-term potentiation reported previously after this chronic treatment (Frow et al., 1990). No changes were seen after the chronic benzodiazepine treatment or the CGP39551, but a range of time intervals and treatments is needed before definitive conclusions can be drawn concerning these compounds.

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247P CENTRAL AND PERIPHERAL (BRAIN AND PLATELET) BENZODIAZEPINE RECEPTORS IN THE RAT - EFFECT OF DIAZEPAM AND CLOBAZAM

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The central benzodiazepine receptor (cBZR) is allied to the GABA receptor complex and affinity of benzodiazepines to this receptor correlates with their activity in certain epilepsy models (Meldrum and Chapman, 1986). 'Peripheral' BZRs are present in many tissues including platelets and brain. Their significance is uncertain, but some pBZR ligands have proconvulsant or anticonvulsant activity (Saano et al, 1989). The effect of benzodiazepine therapy on brain BZR numbers is controversial, (Saano et al, 1989). It is also unclear whether platelet receptors might act as accessable 'markers' for brain effects.

Sprague-Dawley rats (180-240g) were injected i.p. for 4 or 12 consecutive days with diazepam (5mg/kg), clobazam (5mg/kg) or saline control. Animals were sacrificed 16h after the last injection. Blood and dissected cortex of brain were stored at -20°C before batch analysis. cBZRs (in brain), and pBZRs (in brain and platelets) were assayed using [^{3}H]flunitrazepam and PK11195 respectively using modified methods of Pitkanen et al (1987) and Gavish et al (1986). Dissociation constant (Kd) and receptor numbers (Bmax) were calculated both by Scatchard and Saturation Isotherm analysis ($r > 0.95$).

Results in animals treated for four days are shown in Table 1, and were similar at 12 days. Diazepam increased pBZR numbers in platelets after 4 and 12 days (both $P<0.005$) with no change in Kd. No other significant effects were seen.

Table 1: Mean (SEM) Kd (nMol) and Bmax (fmol/mg prot.) of central and peripheral (brain and platelet) BZRs in mice

	n	BRAIN (central)		n	BRAIN ('peripheral')		n	PLATELETS	
		Kd	Bmax		Kd	Bmax		Kd	Bmax
DIAZEPAM	12	1.15 (.05)	1829 (181)	7	1.14 (.39)	461 (128)	11	3.17 (.47)	19,298 (2580)
CONTROL	14	1.27 (.04)	1958 (131)	7	1.01 (.35)	375 (65)	12	3.66 (.53)	11,727 (1173)
CLOBAZAM	5	1.42 (.06)	2134 (252)	5	0.99 (.16)	587 (96)	5	1.86 (.11)	10,884 (1041)
CONTROL	5	1.33 (.05)	1855 (132)	4	0.944 (.07)	628 (67)	5	2.95 (.59)	8,291 (1637)

Diazepam's effect on platelet pBZRs was not reflected in pBZRs or cBZRs in cerebral cortex. Although pBZRs are more numerous in other brain areas, it seems unlikely that platelet pBZRs would be a useful marker for receptor changes in brain. An effect on brain BZRs is not excluded by our results, but, if present, any effect at this dosage is likely to be small.

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248P LACK OF EFFECT OF INTRA-ACCUMBENS DOPAMINE INFUSION ON 5-HT$_3$ RECOGNITION SITES IN LIMBIC AND CORTICAL AREAS OF RAT BRAIN

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5-HT$_3$ receptor antagonists have been shown to suppress the hyperactivity induced by an elevated mesolimbic dopamine function (e.g. Costall et al., 1987). In the present studies we report the effect of dopamine infusion into the nucleus accumbens on the density of 5-HT$_3$ recognition sites and their affinity for 5-HT$_3$ receptor ligands in limbic and cortical areas of the rat brain.

Rats (female, Sprague-Dawley, Bradford strain, 250±25g) received a bilateral infusion of dopamine (50μg 24h^{-1}) or vehicle into the nucleus accumbens for 13 days. Locomotor activity was assessed daily in individual photocell cages. Following locomotor assessment on day 13 animals were killed and the hippocampus, amygdala, frontal and entorhinal cortex dissected out and frozen. Histological assessments were performed to verify cannulae placement and tissue from animals where infusions were outside the nucleus accumbens were discarded from the study. Tissue from 5 animals was pooled and the 5-HT$_3$ receptor recognition site density was determined by saturation experiments (minimum of 6 concentrations, range 0.075-4.0 nM) using [^{3}H]GR65630 (specific activity 64 Cimmol^{-1}, NEN) according to the method of Kilpatrick et al. (1987). Non-specific binding was defined by the inclusion of 10μM granisetron.

Dopamine infused animals produced a significant ($P<0.001$ ANOVA followed by Dunnett's t-test) increase in locomotor activity compared to vehicle infused animals on days 3 and 10 of infusion. However, neither the density of binding sites nor the affinity of the radioligand for the 5-HT$_3$ recognition sites in brain homogenates were signifiantly altered ($P>0.05$ Student's t-test, Table 1).

Table 1. The effect of dopamine/vehicle infusion into nucleus accumbens to modify 5-HT$_3$ recognition sites in rat brain. Values, K_D (nM) and B_{max} (fmol mg^{-1} protein), represent the mean ±s.e.mean, n = 5-8.

	Hippocampus		Amygdala		Frontal cortex		Entorhinal cortex	
	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}
Dopamine	0.7±0.1	30±5	0.7±0.1	61±4	0.5±0.1	32±5	0.5±0.1	45±7
Vehicle	0.4±0.1	27±6	0.5±0.1	54±3	0.5±0.1	30±11	0.5±0.1	41±6

The present studies indicate that after a period of profound changes in behaviour caused by discrete limbic dopamine infusion there was no acute compensatory change to 5-HT$_3$ recognition sites in other limbic or cortical structures. Such receptors may mediate the action of 5-HT$_3$ receptor antagonists to inhibit the behavioural change of dopamine excess.

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249P 5-HT_3- AND 5-HT_4-LIKE ACTIONS OF THE ENANTIOMERS OF ZACOPRIDE IN SOME *IN VITRO* FUNCTIONAL ASSAYS

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Zacopride is a 5-HT_3 antagonist with agonist actions at 5-HT_4 receptors (Dumuis et al., 1989). We have studied 5-HT_3 antagonism by the R(+) and S(-) enantiomers of zacopride, compounds which are reported to differ greatly in potency in some behavioural models predictive of anxiolytic activity (Barnes et al, 1990).

5-HT_3 antagonism was assessed in the rat isolated cervical vagus nerve (RVN) at 27°C using a grease-gap technique to measure 5-HT-evoked depolarization (Ireland and Tyers, 1987) and in the guinea-pig ileum longitudinal muscle - myenteric plexus preparation (GPI) at 37°C where contractions to 5-HT in the presence of methysergide (10^{-6}M) were studied (Buchheit et al, 1985). Non-cumulative dosing with 5-HT was used with a 15 min dosing cycle, agonist contact time was 4 min in the RVN and 1 min in the GPI. The zacopride enantiomers replaced 5-HT in both procedures in studies of their agonist actions. Antagonists were equilibrated for 60 min.

Tissue	pA_2	Racemate	Slope	pA_2	R (+)	Slope	pA_2	S (-)	Slope
RVN	9.1 (8.9-9.3)		1.4 (1.0-1.8)	8.9 (8.3-10.4)		1.0 (0.6-1.5)	9.7 (9.3-11.2)		1.2 (0.6-1.9)
GPI	7.6 (7.3-9.1)		1.2 (0.8-1.7)	7.2 (7.0-7.4)		1.1 (0.9-1.4)	7.6 (7.4-8.0)		1.3 (0.9-1.7)

n = 10-23. 95% confidence limits shown in brackets. 3 or 4 concentrations of antagonist were tested.

Zacopride was a potent 5-HT_3 antagonist in both preparations with the S (-) enantiomer 6 fold more potent than the R (+) in the RVN and 2.5 fold more potent in the GPI. Both enantiomers were surmountable antagonists in the GPI but this was true only of the R (+) form in the RVN. Schild-plot slopes were close to the expected value of unity for competitive antagonists in all cases. Responses to low doses of 5-HT (10^{-9} - 10^{-6}M) in the GPI (5-HT_4 mediated) were markedly reduced by both enantiomers (10^{-7} M or above).

Neither enantiomer (10^{-9} to 10^{-6}M) had agonist actions in the RVN but both evoked contractions of the GPI in the concentration-range 10^{-7} to 10^{-4}M, maximum responses being 20-25% that to 5-HT. The S(-) enantiomer of zacopride evoked 15% of the 5-HT maximum response at 4.3 x 10^{-6}M and was approximately 5 fold more potent than R(+) zacopride. In the presence of ICS 205930 (10^{-6}M) concentration-response curves to both enantiomers in the GPI were displaced to the right with a pA_2 value for ICS 205930 of 6.6 in both cases, consistent with a 5-HT_4 agonist action of the enantiomers, in agreement with Eglen et al., 1990.

Thus, in two in vitro functional assays the enantiomers of zacopride have similar potency as 5-HT_3 antagonists. The compounds also have similar, weak 5-HT_4-like agonist action. These result do not provide evidence for a mechanism underlying the reduced aversive behaviour in rodents in which R(+) zacopride is markedly more potent than the S(-) enantiomer (Barnes et al, 1990). However, in ferrets Middlefell and Price (1991) have described 5-HT_3 agonist effects of S (-) but not R (+) zacopride.

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250P THE ENANTIOMERS OF ZACOPRIDE: ČORRELATION OF ANXIOLYTIC ACTIVITY WITH *IN VIVO* FUNCTIONAL 5-HT_3 ANTAGONISM IN THE MOUSE

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The 5-HT_3 receptor antagonist, zacopride, shows good activity in a range of rodent and primate models of anxiety (Costall et al., 1988). Recently the R(+)-enantiomer of zacopride was reported to be considerably more potent (~10 000 fold) than the S(-)-enantiomer in these models (Barnes et al., 1990). In contrast, in binding studies S(-)-zacopride exhibits slightly greater affinity (~10 fold) than R(+)-zacopride for central 5-HT_3 recognition sites (Barnes et al., 1990). We have compared the activities of zacopride and its enantiomers in models of 5-HT_3 receptor function (von Bezold-Jarisch reflex) and anxiety in the mouse.

Female albino Tuck T/O mice (25-38g; n=4), anaesthetised with urethane (1.5-2.5 g/kg i.p.), were cannulated via the left or right jugular vein, and the cannula advanced so that it lay in, or close to, the right atrium. Heart rate was monitored using ECG leads attached to steel needles through the fore- and hindlegs. A bolus injection of 30-60 µg/kg of 5-HT in 50µl of saline via the jugular cannula evoked the marked bradycardia known as the von Bezold-Jarisch (B-J) reflex as previously reported in the rat (Fozard and Host, 1982). Administration of successive i.v. boluses of 5-HT at 10 min intervals over 60 min produced no significant decrease in the size of the bradycardia. The novel selective 5-HT_3 receptor antagonist, WAY100289 (Rhodes et al., 1991), blocked the B-J reflex by >85% at a minimum effective dose (MED_{85}) of 3 µg/kg s.c. ($P<0.01$;ANOVA). This is comparable to the lowest effective 'anxiolytic' dose of WAY100289 (10 µg/kg s.c.) in the mouse in the light/dark box (Bill et al., 1991). The R(+)- and S(-)-enantiomers of zacopride blocked the B-J reflex with MED_{85}s of 3 and 0.3 µg/kg s.c. respectively, which compares with a MED_{85} of 1 µg/kg for the racemate.

Anxiolytic activity was assessed using the mouse light/dark box (Costall et al., 1988). Mice (n=9) received zacopride or its enantiomers (0.01-1000 µg/kg) or saline s.c. 30 min before being placed individually in the light compartment. Exploration (locomotor activity, rearing and time spent in each compartment) was recorded over the following 5 min.. Saline control mice were markedly less active in the light compartment than the dark, spending 44% of the total time in the light. Light compartment rearing activity (mean +/- s.e.mean per 5min) of saline controls was 15.1+/-1.6 compared to 36.0+/-2.3 for rearing in the dark compartment. Zacopride and its R(+)- and S(-)-enantiomers significantly ($P<0.05$; ANOVA/t-test) increased light compartment rearing (by 40-90%), motor activity (by 40-70%) and time spent in the light (by 10-20%); these effects being similar to those induced by the positive control (chlordiazepoxide 2 mg/kg s.c.). The lowest effective dose (1 µg/kg s.c.) and active dose range (1-1000 µg/kg) were the same for zacopride, R(+)-zacopride and S(-)-zacopride. Thus, using a 10 fold dose-step in the behavioural studies, there was no evidence of a differential 'anxiolytic activity' of R(+)- and S(-)-zacopride using female Tuck mice in the light dark/box. Furthermore, minimally effective doses in the light/dark box correlated reasonably well with the minimum doses required to block the B-J reflex in the mouse after s.c. administration.

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251P EFFECTS OF SUB-CHRONIC TREATMENT WITH CHLORDIAZEPOXIDE, BUSPIRONE AND THE 5-HT$_3$ ANTAGONIST, BRL 46470, ON THE BEHAVIOUR OF MICE

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It has been proposed that the 5-HT3 receptor antagonist, BRL 46470, may be useful for its anxiolytic properties (Upton & Blackburn, 1991). The present studies, comparing its effects with those of chlordiazepoxide and buspirone, show that when given sub-chronically in the drinking fluid it affected behaviour during social encounters and in the light-dark box in a manner which confirmed potent anxiolytic activity.

Each drug was dissolved in the drinking fluid (chlordiazepoxide 21.5 mg/l; buspirone 12.8 mg/l and BRL 46470 40 µg/l) and given for 14 days to pair housed adult male CD1 mice. Average daily intake amounted to 5mg/kg for chlordiazepoxide, 2.4 mg/kg for buspirone and 10 µg/kg for BRL 46470. Control animals received tap water. Effects of the treatments on behaviour were examined in the light-dark box and in the animal's home-cage when encountering an unfamiliar partner. In each case, behaviour was recorded for a 5 min period. The ethological profile described by Dixon (1986) was used to record the acts and postures shown by each mouse during the social encounters.

In the home cage, overall social investigation was significantly increased ($P<0.05$) by each of the drugs (Mean duration ± s. e mean , s.; Controls 71.3 ± 5.9; Chlordiazepoxide 96.9 ± 8.4; Buspirone 95.3 ± 7.6; BRL 46470 117.9 ± 7.0). Occurrence of the specific acts, "Nose", Investigate", "Sniff" and "Follow" was significantly raised by BRL 46470 although not by chlordiazepoxide or buspirone. The enhancement of social investigation by BRL 46470 was associated with a decrease of exploratory behaviour and scanning. There were no increases of social investigation among partners to the drug-treated mice. In the light-dark box, time spent in the light partition was significantly increased ($P<0.05$) by both chlordiazepoxide and by BRL 46470 (Mean duration ± s. e. mean,s; Controls 157.4 ± 9.5; Chlordiazepoxide 195.8 ± 5.9; BRL 46470 183.1 ± 6.6) although not by buspirone (Mean duration ± s. e. mean, s., 166.8 ± 13.9).

It is suggested that the increase of social investigation in the home cage during social interactions provides a sensitive measure of the anxiolytic action produced by sub-chronic administration of all of these compounds. The lack of effect of buspirone in the light-dark box indicates the possibility of a different mechanism of anxiolytic activity from that of the other two compounds. The results seen after sub-chronic treatment in the drinking fluid demonstrate this to be an effective and sensitive means of investigating drug actions and provides additional evidence for the anxiolytic properties of BRL 46470.

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252P EFFECTS OF THE 5-HT$_3$ RECEPTOR AGONIST mCPBG IN THE SOCIAL INTERACTION TEST OF ANXIETY

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The recently developed selective 5-HT3 receptor agonist, 1-(m-chlorophenyl)-biguanide (mCPBG), has been shown to be very potent in *in vitro* preparations (Kilpatrick et al., 1990). However, the effect of mCPBG has not been evaluated using behavioural models. Since 5-HT3 receptor antagonists have been shown to release suppressed behaviour in behavioural tests of anxiety (Jones et al., 1988), the present investigation was designed to investigate the potential anxiogenic effect of mCPBG in the social interaction (SI) test.

Male Lister Hooded rats were implanted with guide cannulae under pentobarbitone anaesthesia to enable the direct injection of mCPBG into the lateral ventricles of the brain (this compound is unlikely to penetrate the brain after peripheral administration). Following a seven day recovery period, rats were injected with either saline or mCPBG (0.001-1.0 µg in 10 µl saline) into the ventricles. Subsequent to injection, animals were paired with an unoperated weight matched (± 30g) partner and behaviours measured in the social interaction test according to the method of Higgins et al (1988). Rats were tested once under high light, unfamiliar (HLU) conditions and seven days later under low light, familiar (LLF) conditions. Locomotor activity was measured by counting the number of line crossings of a 3x3 matrix, drawn on the video monitor during playback. Data were analysed using 2-way ANOVA followed by the Newman Keuls multiple range test.

Analysis of variance revealed that mCPBG had no effect on the SI of rats tested in the HLU environment. In contrast, there was a significant effect of mCPBG in the LLF condition ($F[5,52]=2.7$; $p<0.05$) over the 10min test period. Subsequent post hoc analysis indicated that four doses of mCPBG (0.0001-0.1 µg) significantly reduced ($p<0.05$) rat SI compared with that of saline- treated animals. Thus control animals spent 28.8 ± 4.0s in social interaction whereas rats receiving 0.01 µg mCPBG spent 16.1± 2.1s in interaction. Statistical analysis of the subtrial data showed that the SI of the rats in the LLF test condition significantly decreased over two consecutive 5 minute test periods ($F[1.64]=17.3$; $p<0.01$). Analysis of variance also indicated the existence of a significant effect of mCPBG on SI within the subtrials ($F[5.64]=3.4$; $p<0.05$). Post hoc analysis revealed that, in rats tested under LLF conditions, treatment with the three higher doses of mCPBG (001-1.0 µg) significantly decreased ($p<0.05$) SI during the first 5 minutes of the trial when compared with saline-treated animals. However, drug treatment had no significant effect on animal behaviour during the second 5 minutes of the trial. The reduction in SI was not accompanied by any reduction in locomotor activity at any of the doses tested. Thus under LLF conditions, locomotor activity ranged from 136 ± 12 to 158 ± 7 line crossings.

In conclusion, the 5-HT3 receptor agonist, mCPBG, reduces rat SI under LLF conditions, without affecting locomotor activity, an effect consistent with an increase in anxiety. These data support a modulatory role of 5-HT3 receptors in anxiety.

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253P EFFECT OF ISOLATION REARING ON 5-HT_{1A} AUTORECEPTOR FUNCTION IN THE DORSAL RAPHÉ NUCLEUS

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Rats reared in social isolation display many behavioural and neurochemical changes. Most research into the neurochemical effects of social isolation have focused on the central dopaminergic system; for example, isolates given d-amphetamine show an enhanced behavioural response and increased extracellular dopamine compared to grouped controls (Jones et al., 1988). Recent work looking at the serotonergic system, has found postsynaptic supersensitivity of the 5-HT_{1A} receptor in isolation reared rats (Wright et al., 1991) and it is now well established that the somatodendritic autoreceptor in the dorsal raphe nucleus (DRN) is also of the 5-HT_{1A} subtype. The aim of this study was to determine whether the sensitivity of the somatodendritic 5-HT_{1A} autoreceptor in the DRN was altered in isolation reared rats.

Male Lister hooded rats obtained at weaning (21 days of age) were housed either in isolation or in groups of 4, counterbalanced by weight, for at least six weeks prior to use. Socially or isolation reared rats were anaesthetised with urethane (1.3-1.5 g/kg i.p.) and the jugular vein cannulated for administration of drugs. 5-HT neuronal firing was monitored electrophysiologically using a single barrelled electrode implanted stereotaxically into the DRN. 5-HT neurones were identified by their regular rhythm and slow firing rate (0.5-3.0 spikes/sec) (Aghajanian, 1976) and by their inhibitory response to 8-hydroxy-2-(di-n-propyl-amino)tetralin (8-OHDPAT) a specific 5-HT_{1A} agonist. Basal neuronal firing was not altered between the two groups. 8-OHDPAT was administered to isolation and socially reared animals at a dose (10μg/kg i.v.) shown in an initial study to produce a submaximal inhibition in socially reared rats. 8-OHDPAT (10μg/kg i.v.) produced a greater maximal inhibition of firing (85.1 ± 5.6%, n=6) in isolation-reared animals than in socially-reared (71.7 ± 6.9%, n=6) rats but this difference did not reach significance ($p<0.15$, 2 tailed unpaired Student's t-test). The maximal inhibition occurred within 5 minutes of injection and the duration of the 8-OHDPAT effect lasted for 15-20 minutes in both groups.

The results indicate that the marked functional postsynaptic 5-HT_{1A} receptor supersensitivity observed in isolation reared rats (Wright et al., 1991) does not occur with the somatodendritic 5-HT_{1A} autoreceptor.

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254P EFFECTS OF DIAZEPAM AND THE 5-HT_3 ANTAGONISTS, GRANISETRON AND BRL 46470, ON THE BEHAVIOUR OF INTRUDER MICE

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The 5-HT_3 receptor antagonists have wide ranging psychopharmacological actions, which include anxiolytic activity (Costall et al, 1989). The present experiments, using ethopharmacological procedures, compare the modifications to behavioural responsiveness in intruder mice induced by acute administration of diazepam (DZ) with effects of the 5-HT_3 receptor antagonists, granisetron (GR) and BRL 46470A.

Compounds were administered by stomach tube to intruder male CD1 mice 1h. prior to the behavioural observations. Drug-treated mice (n=15 in each group) received DZ (1, 5 or 10 mg/kg), GR or BRL 46470A (100 ng/kg, 1 μg/kg, 0.1 mg/kg, 1 mg/kg). Controls (n=20) received solvent (1% methylcellulose water). Resident mice had been housed singly 7-11 days prior to the behavioural experiments. Behaviour of each mouse during encounters with an untreated resident in its home cage was recorded by ethopharmacological procedures (Dixon, 1986). The rectal temperatures of the mice were recorded prior to and at the end of each behavioural encounter.

DZ at 5 mg/kg increased the mean duration of social investigation (SI) during aggressive encounters (DZ 58.3 ± 8.8s.; Control 35.6 ± 4s.; $P<0.05$), and it reduced flight in response to the resident's aggression (DZ 6.5 ± 4.7s.; Control 31.3 ± 6.4s.; $P<0.01$). It showed sedative effects at 10 mg/kg (Immobility; DZ 78.7 ± 18.75s.; Control 7.6 ± 1.8s.; $P<0.01$). BRL 46470A at 100 ng/kg increased the mean duration of SI (Treated 59.5 ± 8.8s.; Control 40.3 ± 4.4s.; $P<0.05$). At 0.1 mg/kg, both BRL 46470A and GR decreased the mean duration of retreating relative to the overall flight response of the mice (BRL 46470A 13.2%., GR 10.4%. Control 25.7%; $P<0.05$). No sedative effects were seen. Temperature (°C) (Controls 38.1 ± 0.2) was reduced prior to test by DZ (1mg/kg 37.2 ± 0.2, 5 mg/kg 36.8 ± 0.3, 10 mg/kg 36.3 ± 0.3, all $P<0.05$) and BRL 46470A (0.1 mg/kg 37.4 ± 0.2, 1 mg/kg 37.3 ± 0.2, $P<0.05$). Increase of temperature during encounters was also affected, (Controls +0.48 ± 0.16°C, DZ 1 mg/kg +1.04 ± 0.16, BRL 46470A 0.1 ug/kg + 1.04 ± 0.16, 1 mg/kg + 1.00 ± 0.14).

From the present results it can be seen that 5-HT_3 receptor antagonist, BRL 46470A, demonstrates activities similar to those possessed by DZ in its enhancement of SI, reduction of retreating and influences on temperature. The behavioural effects are indicative of anxiolytic activity. GR did not alter social investigation by the mice or body temperature.

Results expressed as Mean ± S.E.M. Statistics Mann-Whitney U test.

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255P EVIDENCE SUPPORTING AN INVOLVEMENT OF 5-HT_{1C} RECEPTORS IN SOME BEHAVIOURAL EFFECTS OF DOI, AND ITS APPARENT FACILITATION OF 5-HT_{1A} RECEPTOR ACTIVATION *IN VIVO*

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The selective 5-HT2 agonist, DOI (Glennon et al., 1986) has been reported to facilitate the behavioural effects of the 5-HT1A agonists 8-OH-DPAT, buspirone and gepirone (Arnt and Hyttel, 1989; Forster and Fletcher, 1990). These data suggest an important functional interaction between these 5-HT receptor subtypes in vivo. We have examined the effects of several 5-HT antagonists on this interaction, and on the behavioural effects of DOI alone.

DOI (0.5 mg/kg s.c. or greater) markedly increased the potencies of 8-OH-DPAT, buspirone, gepirone and 5-MeODMT to induce the 5-HT behavioural syndrome (forepaw treading, flat posture) in rats. In contrast, the 5-HT2 antagonists (ritanserin, altanserin and mianserin; up to 1mg/kg s.c.) had no effect, whereas BMY 7378 (0.3 mg/kg s.c. or greater), a selective 5-HT1A antagonist/partial agonist (Yocca et al., 1987) significantly antagonised the syndrome. DOI alone (0.5 - 5.0 mg/kg s.c.) induced several components of the '5-HT syndrome' i.e hind-limb abduction forepaw treading and head weaving. These behavioural effects of DOI per se were blocked by mianserin, altanserin and metergoline (ED50 values of 0.4, 0.2 0.03 mg/kg s.c. respectively) but not by ritanserin or BMY 7378 (up to doses of 3 and 1 mg/kg s.c. respectively) . Mianserin and altanserin also antagonised the 'potentiation of the behavioural effects of 8-OH-DPAT induced by DOI. The effect of metergoline, however, was inconsistent - this compound partially antagonised DOI facilitation of 8-OH-DPAT, but at higher doses (0.5 mg/kg s.c. or greater) actually facilitated the syndrome in its own right, possibly indicating that it possesses weak 5-HT1 agonist activity.

The ED50 values for mianserin, altanserin, metergoline and ritanserin to block central 5-HT2 receptors (as determined by antagonism of 5-HTP head twitches in the rat) were 0.004, 0.01, 0.02 and 0.2 mg/kg s.c. respectively, however these potencies do not correlate with the abilities of these antagonists to block the behavioural effects of DOI. DOI also exhibits high affinity for the 5-HT1C receptor subtype (Hoyer, 1988) and it is possible that the activities of the antagonists may correlate more closely with their reported affinities for the 5-HT1C site rather than their 5-HT2 antagonist potencies. In support of this we have found that mianserin, altanserin and metergoline but not ritanserin also antagonise the hypolocomotion induced by MK212 (thought to be 5-HT1C-receptor mediated; Knight and Fletcher, 1989) in the rat, with similar potencies to those for antagonism of DOI.

We conclude that the reported facilitatory action of DOI on the 8-OH-DPAT syndrome may be an additive effect and may be 5-HT1C-receptor mediated.

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256P RU 24969: CHARACTERISATION OF THE LOCOMOTOR RESPONSE IN MICE WITH ISOMERS OF PINDOLOL AND PROPRANOLOL AND EFFECTS ON BRAIN CATECHOLAMINES AND METABOLITES

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RU 24969 produces marked hyperlocomotion in mice which is suggested to result from activation of 5-HT1B receptors and involve catecholaminergic neurones in its expression (Goodwin & Green, 1985; Cheetham et al., 1991). In rats, this response is inhibited by both isomers of pindolol and propranolol (Tricklebank et al., 1986), even though only the (-)enantiomers have high 5-HT1 affinity (Middlemiss et al., 1977). Consequently, we have now characterised RU 24969-induced hyperlocomotion in mice using these isomers. In addition, the effects of RU 24969 on brain catecholamines and metabolites have been determined to further investigate the role of these neurones in this behavioural response.

Antagonists were given 30 min prior to RU 24969 (7.5 mg/kg, ip) to pairs of male C57/Bl/6 mice (20-30 g) and locomotion measured for 60 min with automated photocells. Noradrenaline (NA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations in wholebrain minus cerebellum and dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) concentrations in nucleus accumbens were determined 60 min after RU 24969 (3.75-10 mg/kg, ip) by HPLC-ECD.

Table 1 Effects of RU 24969 on brain catecholamines and metabolites.

		NA	MHPG	DA	DOPAC	HVA	3-MT	DOPAC/DA
Saline		514 ± 7	89 ± 2	5899 ± 184	767 ± 88	785 ± 40	138 ± 17	0.129 ± 0.012
RU 24969	3.75	508 ± 4	102 ± 3*	5855 ± 208	783 ± 78	809 ± 19	116 ± 10	0.133 ± 0.010
(mg/kg, ip)	7.5	515 ± 12	104 ± 4**	5819 ± 143	770 ± 59	861 ± 33	117 ± 11	0.134 ± 0.013
	10	492 ± 10	114 ± 5**	5904 ± 322	877 ± 50	866 ± 33	100 ± 8*	0.151 ± 0.012

Values (ng/g wet weight) ± s.e. mean, n = 6 - 8. Significantly different from saline *P<0.05, **P<0.01, Williams' test.

(-)Pindolol (4 mg/kg, ip) and (-)propranolol (20 mg/kg, ip) markedly attenuated the increased hyperlocomotion induced by RU 24969 by 33% (P<0.001) and 62% (P<0.001) respectively. In contrast, the (+)isomers were without effect. The differential actions of these isomers on RU 24969-induced hyperlocomotion provides substantial evidence for the 5-HT1 mediation of this response. RU 24969 increased brain MHPG (Table 1) and this is consistent with the involvement of noradrenergic systems and the inhibition of the hyperlocomotion by prazosin (Cheetham et al., 1991). However, no parallel increase in dopamine turnover was observed (Table 1) to account for the susceptibility of this response to D1 or D2 receptor antagonists (Cheetham et al., 1991).

Overall, the data provide additional evidence that RU 24969-induced hyperlocomotion in mice is a 5-HT1-mediated response and, furthermore, that noradrenergic neurones are involved in its behavioural expression.

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257P CONSEQUENCES OF PHOTOCHEMICALLY-INDUCED ISCHAEMIA ON 5-HT BIOCHEMISTRY IN THE RAT BRAIN

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Abnormal neurotransmitter function may contribute to the neurodegeneration following cerebral ischaemia. This study investigated the effect of photochemically induced ischaemia (Watson et al, 1985) on 5-HT biochemistry in the rat cortex.

Male Lister hooded rats (250-350g) were anaesthetised with halothane and given Rose Bengal (20mg kg^{-1} i.v.). The right skull was illuminated with a green light (8mm diameter; 150W halogen) for 7 min. Rats were killed at various times and the right cortex dissected. 5-HT concentrations were measured by h.p.l.c. (Green et al, 1990). 5-HT synthesis rates were measured 1h after giving tranylcypromine (10mg kg^{-1} i.p.). Neuronal 5-HT uptake was measured by incubating homogenates with [^{3}H]-5-HT. Binding to 5-HT_{1A} and 5-HT_2 receptors were measured using [^{3}H]-8-OHDPAT and [^{3}H]-ketanserin.

Time post-ischaemia:		sham	3h	24h	7days
5-HT concentration	(nmol g^{-1})	0.41±0.08	0.42±0.09	-	0.39±0.05
5-HIAA concentration	(nmol g^{-1})	0.89±0.07	1.20±0.11*	-	0.73±0.07
5-HT synthesis	(nmol g^{-1} h^{-1})	1.00±0.08	0.55±0.08**	-	0.72±0.08*
[^{3}H]-5-HT uptake	(pmol g^{-1} h^{-1})	789±122	-	-	409±51 *
[^{3}H]-8OHDPAT binding	(pmol g^{-1})	1.98±0.10	-	0.66±0.10**	1.17±0.10*
[^{3}H]-ketanserin binding	(pmol g^{-1})	3.00±0.40	-	1.08±0.20**	2.04±0.20*

Mean ± s.e.mean. Significantly different from sham: *p<0.05; **p<0.01 (t-test or ANOVA).

Results are shown in the table. 5-HT levels were unchanged but 5-HIAA was elevated 3h post-ischaemia. The rate of 5-HT synthesis was reduced 3h and 7 days after ischaemia. Uptake was reduced 7 days post-ischaemia and binding to 5-HT_{1A} and 5-HT_2 receptors reduced both 1 and 7 days later. Thus, 5-HT biochemistry is markedly affected by an ischaemic insult to the rat cortex. The role 5-HT and other neurotransmitters may play in mediating the neurodegeneration following ischaemia remains to be determined.

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258P COMPARISON OF AMPA AND IBOTENIC ACID LESIONS IN RAT CORTEX

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In our previous study employing ibotenic acid and 5,7-DHT lesions (Lawrence et al, 1991), no evidence was found for a presynaptic localisation of $5HT_{1A}$ binding sites on cholinergic or serotonergic nerve terminals in the rat cortex or hippocampus.

This study has now been extended to include a unilateral excitotoxic lesion with the selective quisqualate receptor agonist, AMPA ((RS)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and to compare this lesion with that of ibotenic acid at the $5HT_{1A}$ site, labelled with [^{3}H]8-OHDPAT (0.5nM).

Cholinergic neurones in the substantia innominata were destroyed by direct injection of either ibotenic acid (25nmoles) or AMPA (7.5nmoles; AP - 0.9mm; ML ± 2.6mm; DV - 6.8mm). The lesions were verified by measurement of cortical and hippocampal levels of choline acetyltransferase (ChAT) after 7 days.

Table 1: ChAT activity and binding to $5HT_{1A}$ receptors following ibotenic acid and AMPA lesions

	Ibotenic acid (n = 6)		AMPA (n = 6)	
	control	lesion	control	lesion
CORTEX				
ChAT activity (nmoles/mg protein/60 min)	54.4 ± 3.9	27.3 ± 3.1	46.5 ± 4.1	15.1 ± 1.7
8-OHDPAT binding: K_D (nM)	1.38 ± 0.06	1.38 ± 0.06	1.21 ± 0.30	0.92 ± 0.21
B_{max} (pmol/mg protein)	0.45 ± 0.02	0.52 ± 0.03	0.49 ± 0.03	0.47 ± 0.05
HIPPOCAMPUS				
ChAT activity (nmoles/mg protein/60 min)	61.3 ± 5.8	62.3 ± 5.2	56.5 ± 5.5	55.9 ± 5.3
8-OHDPAT binding: K_D (nM)	1.51 ± 0.14	1.28 ± 0.08	1.11 ± 0.23	1.33 ± 0.11
B_{max} (pmol/mg protein)	1.83 ± 0.06	1.85 ± 0.14	1.61 ± 0.02	1.72 ± 0.03

Thus ibotenic acid and AMPA produced a similar level of neuronal destruction in the substantia innominata, as measured by the ChAT activity. However, for both lesions no changes were observed in either the density or affinity of $5HT_{1A}$ receptors in the cortex. This data confirms our previous observation that $5HT_{1A}$ receptors are not located on cholinergic terminals in the rat cortex.

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259P GR68755, A POTENT AND SELECTIVE ANTAGONIST OF 5-HT$_3$ RECEPTORS

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Selective 5-HT$_3$ receptor antagonists are clinically effective in treating emesis resulting from cancer chemotherapy or radiation therapy (see Kilpatrick et al., 1990). This class of compound may also be effective in several psychiatric disorders (Costall et al., 1990). We now report on the pharmacological profile of a novel 5-HT$_3$ receptor antagonist, GR68755 (2,3,4,5-tetrahydro-5-methyl-2-[(5-methyl-1H-imidazol-4-yl)-methyl]-1H-pyrido[4,3-b]indol-1-one) maleate.

When examined *in vitro*, GR68755 was a potent and selective antagonist at 5-HT$_3$ compared with other 5-HT receptors (Table 1). GR68755 (10μM) also had no effect on α_1, β_1, β_2 adrenoceptors; muscarinic M_1, M_2, M_3 receptors; dopamine D_2 receptors; $GABA_A$ receptors or neuronal nicotinic receptors.

Table 1 The affinities of GR68755 in several 5-HT receptor containing preparations.

Isolated tissue	Agonist/radioligand	Receptor	pA_2 or pK_i
Rat vagus nerve	5-HT	5-HT$_3$	9.8*
Rat entorhinal cortex	[^{3}H]GR65630	5-HT$_3$	9.4
Rat aorta	5-HT	5-HT$_2$	5.2
Dog saphenous vein	5-HT	5-HT$_1$-like	<5
Rat hippocampus	[^{3}H]8-OH-DPAT	5-HT$_{1A}$	<4

* Decrease in maximum agonist response observed, a pK_B value is presented with concentration ratios calculated according to Kennedy & Roberts, 1985. Other methods are quoted in Butler et al., 1988 and Kilpatrick et al., 1987.

In the anaesthetised cat, 5-HT$_3$ receptor agonists induce the Bezold Jarisch reflex (BJR). GR68755 (0.1-1μg/kg i.v.) caused a dose-related inhibition of the 2-methyl-5-HT (3-50μg/kg) induced BJR. The onset of action was rapid (<1 min) and the duration of effect was dose-related. GR68755 (3-10μg/kg) was also active when administered by the intraduodenal route, with a duration of action of >3 hours at 3μg/kg. GR68755 at doses of 2.5mg/kg i.v. and below caused no overt behavioural effects in the mouse, rat and dog. At a dose of 1mg/kg i.v., no cardiovascular effects were observed in the anaesthetised cat.

We conclude that GR68755 is a potent, long-lasting and selective 5-HT$_3$ receptor antagonist.

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260P FREQUENCY DEPENDENT DOPAMINE EFFLUX FROM THE NUCLEUS ACCUMBENS IN RAT BRAIN SLICES: THE INFLUENCE OF AUTORECEPTORS

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Fast cyclic voltammetry has been used to measure evoked dopamine (DA) efflux from rat brain slices (350 μm), superfused with artificial cerebrospinal fluid at 32°C (Palij *et al.*, 1990). Evoked DA efflux in the caudate-putamen (CPu) and the tuberculum olfactorium (TuO) has been shown to differ with respect to frequency of stimulation by trains of 20 pulses, relative to that evoked by a single pulse (Trout *et al.*, 1991). In contrast to the largely A9-innervated CPu, efflux of DA evoked by trains of 20 pulses showed a strong frequency dependence in the TuO (Trout *et al.*, 1991).

The nucleus accumbens (NAc) receives its dopaminergic innervation predominantly from fibres originating in the A10 region. The frequency-response relationship in the NAc has been investigated and compared with that in the TuO to see if DA release in these regions is characteristic of innervation derived from the ventral tegmental area.

DA efflux in the NAc is frequency-dependent, like that previously reported in the TuO (Trout *et al.*, 1991) and shown here (Figure 1). 50 Hz appears to be an optimum frequency for this train length in both regions. In the TuO, autoinhibition appears to account for the fall in efflux at 10 and 20 Hz since it is blocked by metoclopramide (0.3 μM). Metoclopramide also significantly increased evoked DA efflux at 10 and 20 Hz in the NAc, also to a similar level to that observed at 50 Hz in this region.

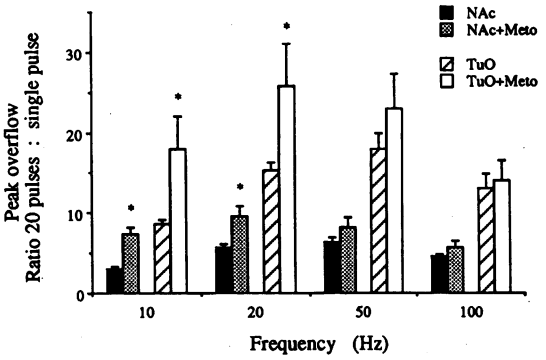

Figure 1 Effect of frequency on DA efflux evoked by 20 pulse trains in NAc & TuO in the presence and absence of metoclopramide (Meto, 0.3 μM). * $P < 0.05$ ($n \geq 5$), control compared to Meto for each area.

Autoinhibition appears to operate under similar conditions in these two areas, but the amount of DA efflux evoked by trains of 20 pulses, relative to a single pulse, is greater in the TuO than in the NAc. Other, so far unknown factors may also affect the efflux of DA during trains of electrical pulses.

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261P THE RELATIONSHIP BETWEEN THE EXTENT OF NIGRAL LESION INDUCED BY QUINOLINIC ACID AND ROTATIONAL BEHAVIOUR IN RATS

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Parkinson's disease manifests when over 80% of dopamine-containing cells in the substantia nigra have degenerated (Bernheimer et al., 1973). Few reports have investigated the link between the destruction of nigral cells and rotational assymmetry in rats. We sought to establish a predictive model of cell loss in the substantia nigra of rats using dopamine agonist-induced rotation as an index.

Quinolinic acid (25-300nmol in 1µl) was unilaterally injected into the substantia nigra pars compacta (SNc) using standard stereotaxic procedures. Phosphate buffered saline (10mM) was injected at an indentical locus in the contralateral hemisphere to act as control. Seven days post surgery rats were tested for the rotational behaviour 12 min after apomorphine (0.5mg/kg, s.c.) or 32 min after (+)-amphetamine (5.0mg/kg, i.p) challenge. Animals were then killed and the loss of cells in the SNc injected with quinolinic acid expressed as a percentage of controls determined in Nissl stained brain sections. In a separate experiment the loss of tyrosine hydroxylase (TH)-immunoreactive cells in the SNc was assessed following intranigral injection of quinolinic acid (25-150nmol).

Quinolinic acid decreased the percentage of cells remaining in the SNc as measured by Nissl stain ($F = 31.4_{(3,24)}$ $p < 0.001$) and TH-immunoreactivity ($F = 12.5_{(3,17)}$ $p < 0.001$) in a concentration-dependent fashion. There was no difference ($F = 1.80_{(1,35)}$ $p > 0.05$) between the cell depletion as measured by Nissl stain or TH-immunoreactivity. The percentage of Nissl stained cells remaining in the lesioned SNc was correlated with the number of ipsilateral rotations induced by apomorphine but not (+)-amphetamine (Figure 1).

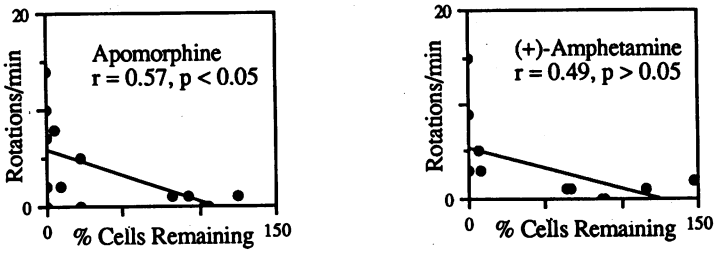

Figure 1. Correlation Between Ipsilateral Rotation Induced by Apomorphine and (+)-Amphetamine and the Percentage of Cells Remaining After Quinolinic Acid Lesion.

These results demonstrate that apomorphine-induced rotation manifests in the rat when over 75% of dopamine containing cells in the SNc are lost, whilst (+)-amphetamine-induced rotation appears only with near total destruction. Therefore, the apomorphine- but not the (+)-amphetamine-rotating rat models the loss of nigral neurones observed in Parkinson's disease.

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262P REDUCTIONS IN FORSKOLIN AND D_1-DOPAMINE RECEPTOR BINDING FOLLOWING FOCAL CEREBRAL ISCHAEMIA IN THE RAT

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The changes in second messenger systems that occur after cerebral ischaemia (Tanaka et al., 1991) may be involved in the pathogenesis of ischaemic damage. We have studied the effects of permanent occlusion of the left middle cerebral artery (MCA) on forskolin and D_1 dopamine receptor binding in the rat. Quantitative autoradiographic techniques were used to determine ligand binding and local cerebral blood flow (CBF) within the same animals.

Male Sprague-Dawley rats were subjected to permanent left MCA occlusion (Tamura et al. 1981) and killed 2 hours (n=6) or 24 hours (n=4) following occlusion. Twenty µm coronal sections were cut through the cerebrum for autoradiography. D_1 dopamine (using the specific ligand SCH23390) and forskolin binding were measured using the methods of Savasta et al. (1986) and Gehlert et al. (1985) respectively. Local CBF was evaluated in adjacent tissue sections using the [^{14}C]-iodoantipyrine technique (Sakurada et al., 1978).

Two hours after occlusion, CBF was significantly reduced throughout the left MCA territory (Table 1). Despite this widespread hypoperfusion, forskolin and SCH23390 binding were significantly reduced only in the dorsolateral caudate nucleus. Twenty four hours after the onset of ischaemia forskolin binding was also significantly reduced in the frontal cortex (Table 1).

Table 1

	CBF 2h (ml^{-1} $100g^{-1}$ min)		^{3}H Forskolin 2h		^{3}H Forskolin 24h		^{3}H SCH23390 2h		^{3}H SCH23390 24h	
	Contra.	Ipsi.	Contra.	Ipsi.	Contra.	Ipsi.	Contra.	Ipsi.	Contra.	Ipsi.
Frontal Cortex	156±23	37± 4*	67±11	68±13	88± 4	37±20*	9± 4	8± 3	16± 3	12± 1
Ant.Cing. Cortex	186±14	111±14*	95±14	99±16	87± 5	87± 7	16± 4	18± 3	19± 2	20± 2
Caudate -dl	166±23	9± 5*	215±30	87±11*	238±31	21±11*	139±41	110±31*	147±15	103± 7*
Caudate -vm	158±13	102± 5*	210±29	220±33	189±12	197±15	141±31	135±32	152±23	158±19

Ant. cing.: anterior cingulate; dl: dorsolateral; vm: ventromedial. Values are mean ± s.e. mean contralateral (Contra.) and ipsilateral (Ipsi.) to occlusion. Binding values are $pmol^{-1}g$ tissue. Significant difference from contralateral value: *P<0.05 (paired t-test, Bonferroni correction).

These results demonstrate alterations in signal-transduction mechanisms at both the second messenger and neurotransmitter receptor level after 2 and 24 hours of focal cerebral ischaemia. The neuroanatomical areas with the largest falls in blood flow - frontal cortex and dorsolateral caudate - were the only areas in which significant changes in forskolin and D_1 receptor binding were observed. This suggests blood flow must fall below a certain threshold in order to cause alterations in binding. In the dorsolateral caudate nucleus the ischaemic threshold for reduction in forskolin binding sites was determined as $34ml^{-1}100g^{-1}$ min.

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263P 6-HYDROXYDOPAMINE INJECTED INTO THE LATERAL HYPOTHALAMUS CAUSES A FUNCTIONAL DEPLETION OF DOPAMINE IN THE RAT CAUDATE PUTAMEN AND NUCLEUS ACCUMBENS

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A depletion of dopamine (DA) in the rat striatum is essential if tissue implants are to be considered as models for therapy for Parkinson's Disease. Here we describe, for the first time a functional depletion of DA in the rat striatum using Fast Cyclic Voltammetry (FCV). Five female Wistar rats anaesthetised with chloral hydrate (500mg/kg i.p.) received stereotaxic injections of 8μg 6-hydroxydopamine HCl (6-OHDA) dissolved in 4μl 0.9% w/v NaCl into the right lateral hypothalamus (V=1.4-1.6mm, L=1.6mm, AP=4.7mm, Paxinos & Watson, 1982); the contralateral side acted as the control. One week later the animals were given 2mg/kg (i.p) (+)-amphetamine sulphate; these showed ipsilateral turning behaviour of 26.8±3.06 turns per minute. Two days later DA overflow was measured by FCV (Bull *et al.* 1990) in a contralateral and ipsilateral brain slice containing the caudate putmen (CPu) and nucleus accumbens (ACb). Three single pulses (0.1ms, 20V) and subsequently three trains of 20 pulses at 50Hz. were delivered separately to the CPu and ACb. Table 1. shows the mean amount of DA overflow (μM) evoked by the stimuli.

Table 1: DA overflow (μM) evoked by stimuli in lesioned and non-lesioned nuclei

Non-Lesioned CPu		Lesioned CPu		Non-Lesioned ACb		Lesioned ACb	
Single Pulse	Train	Single Pulse	Train	Single Pulse	Train	Single Pulse	Train
0.216 (±0.029)	0.325 (±0.081)	0*	0.02 (±0.02)*	0.256 (±0.064)	1.89 (±0.486)	0.03 (±0.03)*	0.2 (±0.155)*

* P=<0.01 when compared to the non-lesioned nucleus. (mean (±SEM); n=5)

The results show that unilateral injection of 6-OHDA into the lateral hypothalamus reduces dopamine overflow in the ipsilateral CPu and ACb. DA overflow in non-lesioned CPu or ACb evoked by single pulses or a train of 20 pulses at 50Hz was not significantly different from that reported by O'Connor *et al.* (1991). Overflow following single pulse stimulation was undetectable in the lesioned CPu, and reduced to 11% in the ACb. By contrast, trains of stimuli in the ACb resulted in approximately 10% overflow while in the CPu overflow to such stimulation was 6% of the contralateral side. Further studies are needed to establish reasons for the differences observed. Approaches may include, more extensive lesions in the lateral hypothalamus, selective lesions in the A8, A9 and A10 regions, comparison of dopamine release following unilateral injections of saline, and biochemical estimates of dopamine content in the ACb and CPu.

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264P CONTRASTING EFFECTS OF DOPAMINE D_1 AND D_2 RECEPTOR AGONISTS ON OPIOID PRECIPITATED WITHDRAWAL

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The behavioural signs of morphine withdrawal are thought to be partially mediated through a supersensitive dopamine (DA) receptor system (Ary et al., 1977; Lal, 1976). It is possible that the expression of morphine abstinence may be differentially modulated by selective activation of DA D_1 or D_2 receptor subtypes. The current study describes the effects of the selective agonists SFK38393 (D_1) and quinpirole (D_2) on naloxone-precipitated morphine withdrawal.

Male Wister rats (UWCC) were employed throughout (n=12). Animals received twice daily injections of morphine on an increasing schedule starting with 20mgkg^{-1} on day 1 up to 200mgkg^{-1} by day 10. SKF38393 (5mgkg^{-1} sc) or quinpirole (5mgkg^{-1} sc) were administered 30 mins prior to naloxone (5mgkg^{-1} ip)-precipitated withdrawal. Abstinence signs were recorded in pairs of rats for 30 mins and body temperature and weight measured up to 2 hrs post naloxone.

SKF38393 was without effect on the behavioural expression of withdrawal but exacerbated acute body weight loss (+18.6%; P<0.05). Though quinpirole markedly increased (P<0.01) locomotor (+493%) and scored aggressive behaviour (+104%), it reduced other withdrawal signs which included: acute body weight loss (-17.4%), incidence of wet dog shakes (-67.8%), burrowing (-85.4%), vacuous chewing (-100%), head weaving (-100%) and hypothermia (-77.8%). In corresponding control temperature studies, quinpirole was inherently hypothermic (-1.9^{o}C; P<0.01) whilst SKF38393 was devoid of effect.

The results suggest that the intensity of morphine withdrawal behaviours can be attenuated by D_2 receptor stimulation though aggressive and locomotor behaviours are concurrently unmasked. It might be additionally suggested that some quinpirole induced effects during opioid withdrawal are eclipsed by its own inherent activity. Thus, even though D_2 receptor agonism evokes temperature loss, withdrawal hypothermia is attenuated possibly as a secondary consequence of increased locomotor activity.
The authors wish to acknowledge Dept. of Education, N. Ireland.

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265P ATTENUATION OF LOCOMOTOR STIMULANT RESPONSES TO NICOTINE AND AMPHETAMINE BY SELECTIVE DOPAMINE RECEPTOR ANTAGONISTS

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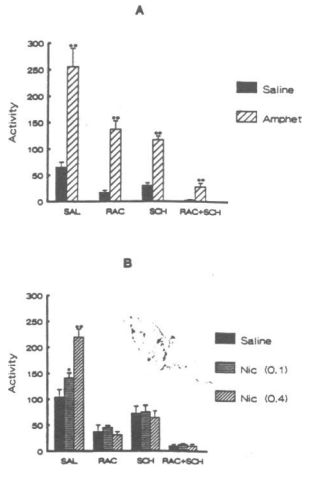

Figure 1: The effects of DA receptor antagonists on locomotor responses to amphetamine and nicotine. Data are expressed as means ± SEM of 6 results. Significantly greater than rats treated with SC saline; *P < 0.05; **P < 0.01. (Duncan's test).

Although it is generally accepted that the locomotor stimulant properties of nicotine (NIC) and d-amphetamine (AMPHET) are related to their effects on mesolimbic dopamine (DA) secretion (Clarke 1990; Wise & Bozarth 1987), little appears to be known of the DA receptor subtypes which mediate the responses. In this study the selective DA_1 antagonist, SCH 23390 (SCH), and DA_2 antagonist, raclopride (RAC), have been used to address this issue.

In the studies with AMPHET, male Sprague-Dawley rats (N = 6 per group) were given intraperitoneal (IP) injections of saline (SAL), RAC (0.15 mg/kg), SCH (0.01 mg/kg), RAC or SCH+RAC followed 10 minutes later by a subcutaneous (SC) injection of SAL or AMPHET (0.3 mg/kg). Locomotor activity was measured automatically in an activity box during a 16 minute trial starting 30 minutes after the SC injection. A similar protocol was used to examine the effects of the antagonists on the responses to NIC (0.1 and 0.4 mg/kg) which was given 5 minutes pre-trial (35 minutes after the IP injection) to animals which had been sensitised to its stimulant effects by pretreatment with the drug for 16 days prior to the first test day (Clarke 1987). Both AMPHET and NIC increased ($P < 0.01$) the activity of the rats pretreated with IP saline (Fig. 1). Pretreatment with SCH and RAC decreased the activity of rats given SC saline ($P < 0.01$), AMPHET ($P < 0.05$) or NIC ($P < 0.01$). The activity was decreased further ($P < 0.01$) by the co-administration of both antagonists. Pretreatment with the antagonists abolished the response to NIC (Fig. 1B) whereas that to AMPHET remained significant ($P < 0.01$) (Fig. 1A). The data suggest that the locomotor responses to NIC and AMPHET depend upon stimulation of DA_1 and DA_2 receptors and that the responses to NIC appear to be more sensitive to the effects of the antagonists.

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266P THE INFLUENCE OF ADRENALECTOMY ON THE DESENSITISATION OF α_2- AND β-ADRENOCEPTORS INDUCED BY REPEATED ELECTROCONVULSIVE SHOCK

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Although the mechanism for the adaptive response of central adrenoceptors to repeated electroconvulsive shock (ECS) remains unclear, it has been shown that noradrenaline release is probably not involved because down-regulation of postsynaptic alpha2- and ß-adrenoceptors occurs after noradrenergic denervation (Dooley et al., 1987; Heal et al., 1991). However, prolonged injection of adrenocorticotrophic hormone (ACTH) down-regulates ß-adrenoceptor-stimulated adenylate cyclase in rat cortex (Duman et al.,1985) and electroconvulsive therapy (ECT) produces marked increases in plasma corticosteroids (see Beuret & Swanson, 1969). Since this raises the possibility that ECS produces some of its effects by increasing ACTH release, we have determined whether adrenalectomy modifies the effects of ECS on alpha2- and ß-adrenoceptors in rat brain.

Adrenalectomised (ADREN) and sham-operated (SHAM) CD rats (100 - 120 g), purchased from Charles River (Margate), received an ECS (200V, 2s) or halothane 5 times over 10 days. Pre- and postsynaptic alpha2-adrenoceptor function was respectively assessed using clonidine (0.1 mg/kg, ip) hypoactivity (rated 0-3 on 4 parameters at 10 min intervals for 60 min) and clonidine (0.03 mg/kg, ip) mydriasis (measured at 10 min). Cortical ß-adrenoceptors were measured using [3H]-dihydroalprenolol (0.07-3.6 nM) with total ß- and ß1-adrenoceptors defined by isoprenaline (200 µM) and CGP 20712A (100 nM) respectively.

Table 1 Effect of ECS on alpha2- and ß-adrenoceptors in adrenalectomised and sham-operated rats.

Treatment group	Hypoactivity score	Mydriasis (mm)	Total ß (fmol/mg protein)	ß1 (fmol/mg protein)
SHAM/HALO	27.4 ± 0.7	0.24 ± 0.01	139 ± 4	89 ± 4
SHAM/ECS	23.1 ± 0.9**	0.16 ± 0.01***	121 ± 3**	73 ± 1***
ADREN/HALO	27.9 ± 1.2	0.24 ± 0.02	141 ± 5	91 ± 4
ADREN/ECS	21.7 ± 1.0**	0.16 ± 0.02**	128 ± 2*	77 ± 2**

Results are mean ± s.e. mean, n=8-11. Significantly different *P<0.05, **P<0.01,***P<0.001.

As shown in Table 1, adrenalectomy did not modify pre- and postsynaptic alpha2-adrenoceptor function or ß-adrenoceptor number. Adrenalectomy also had no effect on the attenuation of clonidine hypoactivity and mydriasis induced by repeated ECS and, similarly, did not modify the reduction in total ß- and ß1-adrenoceptors produced by this treatment. The results suggest adrenal hormones probably have no role in the actions of ECS and are therefore consistent with the reports that ECT is effective in patients who are devoid of adrenal function (see Beuret & Swanson, 1969).

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267P ANTAGONISM OF NEFOPAM ANTINOCICEPTION BY NALOXONE AND RX821002

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Nefopam is an effective analgesic in man (Heel, et al 1980). It's mechanism of action remains unclear. It has, however been shown that nefopam does not bind to opioid receptors and does not inhibit cyclo-oxygenase activity (Conway & Mitchell, 1977). A feature of nefopam's pharmacology is that it blocks the uptake of monoamines, thus resembling the tricyclic antidepressants (TCA). We have shown that TCA induced antinociception is sensitive to opioid antagonist blockade (Gray et al, 1990) To extend these studies we have investigated the antinociceptive profile of nefopam in the mouse abdominal constriction (AC) test.

Treatment ($mgkg^{-1}$)	% Protection
Nefopam 5	62.32±2.77
+Naloxone 0.5	26.20±2.49*
+Prazosin 1	68.84±4.16
+RX821002 1	49.20±2.74*
Nefopam 15	96.11±1.37
+Naloxone 0.5	67.19±3.97*
+Prazosin 1	93.25±2.80
+RX821002 1	74.62±3.34*
*P<0.05-v-Nefopam	

Male ICI-GB1 mice (20±2g; n=8) were pretreated with nefopam (sc) 30 mins and naloxone ($0.5mgkg^{-1}$sc) 5 min before 1% acetic acid challenge; alternatively, prazosin or RX821002 were co-administered (both at $1mgkg^{-1}$sc) with nefopam. Abdominal constrictions were evaluated over 20 min. Nefopam produced protection from acetic acid induced-AC (see table), and prazosin did not antagonise this activity. There was, however, significant attenuation when nefopam was combined with either the α_2 adrenoceptor antagonist RX821002 (Hudson & Nutt, 1990) or naloxone. Thus, these findings strongly suggest that increased intrasynaptic levels of NA may interact with postsynaptic α_2 adrenoceptors as one of the major neuronal loci for nefopam antinociception. Moreover, since nefopam antinociception is naloxone reversible, there appears to be a co-involvement of opioidergic and α_2 adrenoceptor mechanisms in nefopam antinociceptive (AC) action.

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268P BEHAVIOURAL PROFILE OF THE NON-ADRENOCEPTOR IDAZOXAN BINDING SITE LIGAND RX801023 IN MICE

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Idazoxan binds with almost equal high affinity to both α2-adrenoceptors and to non-adrenoceptor idazoxan binding sites or NAIBS (Michel & Insel, 1989). RX801023 (6-fluoro-2-(2-(1,4-benzodioxanyl)-2-imidazoline) is an analogue of idazoxan which, in receptor binding studies, binds with high affinity to NAIBS, but has over 10-fold lower affinity at α2-adrenoceptors (Mallard et al., 1991). In this study we have determined the behavioural profile of RX801023 in male TO mice (25-35g; n= 6-10) using the following paradigms: observational analysis; rotarod performance; locomotor and head-dipping behaviour in automated activity boxes; body temperatures and electroshock seizure threshold (Jackson et al., 1990). RX801023 was dissolved in saline and administered i.p.

RX801023 (0.3, 3, 30 mg/kg) had no effect on rearing, wall-climbing, scratching, gnawing, sniffing, yawning or stereotyped mouthing over a 30 (or 60 min) observation period but produced a significant decrease in grooming at the 30 mg/kg dose (score of 1.0±0.4 vs. 7.7±2 for vehicle at 30 min; maximal score of 30). No unusual behaviours were detected. RX801023 (3-30 mg/kg) did not significantly alter rotarod performance and in doses of 1, 3 and 10 mg/kg had no effect on locomotion in mice placed in a novel environment. However, RX801023 10 mg/kg significantly decreased locomotion in animals habituated to the activity boxes for 40 min before injection (cumulative counts at 30 min: vehicle 6000±1262; drug-treated group 2459±564). Moreover, hole-dipping - a measure of exploration - was significantly decreased by RX801023 (3, 10 mg/kg) in both habituated (cumulative counts at 30 min: vehicle 307±66; 3 mg/kg 137±35; 10 mg/kg 55±28) and unhabituated mice (cumulative counts at 30 min: vehicle 449±30; 3 mg/kg 334±38; 10 mg/kg 162±35) although again effects were more marked in the habituated animals. RX801023 (0.3, 3 mg/kg) had no effect on body temperature. A higher dose (30 mg/kg i.p.) produced significant hypothermia 30 min after injection (37.0±0.2 vs 35.0±0.3). Finally, RX801023 (0.3, 3, 30 mg/kg i.p.) had no effect on electroshock seizure threshold in mice. Thus CD50 values (current producing seizures in 50% of the sample) and 95% fiducial limits were: vehicle 10.5 (9.7-11.4); 0.3 mg/kg 11.2 (10.3-12.2); 3 mg/kg 10.9 (10.0-11.9); 30 mg/kg 11.3 (10.1-12.7). Hence, the behavioural effects of RX801023 in mice show some clear differences from those of selective α2-adrenoceptor antagonists which, for example, are pro-convulsant (Jackson et al., 1990). These preliminary findings of the behavioural effects of a ligand with increased selectivity for NAIBS over α2-adrenoceptors do not provide any major clues towards a possible physiological role for NAIBS. However, the decrease in head-dipping induced by relatively low doses of RX801023 in both habituated and unhabituated mice is interesting, and suggests central actions of this drug that may be worthy of further investigation.

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269P THE EFFECT OF GUANINE NUCLEOTIDES ON [^{3}H]-IDAZOXAN BINDING SITES IN BOVINE CEREBRAL CORTEX

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Imidazoline compounds such as idazoxan bind not only to α_2-adrenoceptors but to other high affinity sites in a variety of tissues and species, e.g. the so called "non-catecholamine, idazoxan binding sites" in guinea-pig brain membranes (Wikberg and Uhlen, 1990). In this study we have examined the effects of GTP on ^{3}H-idazoxan binding to bovine cerebral cortex membranes, in the presence and absence of yohimbine, in an attempt to determine whether the non-α_2-adrenoceptor binding site might represent receptors belonging to the super-family associated with guanine nucleotide binding proteins.

Membranes were incubated with 1nM ^{3}H-idazoxan in Tris buffer (pH 7.4) for 90 mins at room temperature in a total volume of 0.5ml. Bound radioactivity was separated by rapid filtration and non-specific binding determined in the presence of 1μM cirazoline. The selective α_2-antagonist yohimbine competed with ^{3}H-idazoxan in a biphasic manner (Hill slope=-0.72) which was resolved by a non-linear curve fitting programme (Graphpad) into two sites. The higher affinity site (presumed to be α_2-adrenoceptors) comprised 20% of total binding with a pK_i of 9.2±0.2 (n=3) and the other (non-α_2-adrenoceptor) had a pK_i of 7.3±0.02. A concentration of 50nM yohimbine (80 fold greater than its K_i for α_2-adrenoceptors) was therefore used to mask binding to α_2-adrenoceptors. Saturation curves were then constructed using increasing concentrations of ^{3}H-idazoxan in the presence and absence of 50nM yohimbine (Yoh) and GTP (0.3mM). The B_{max} values in the presence of GTP are significantly different ($p< 0.05$) (unpaired t-test) from those without GTP (see Table). GTP also reduced the affinity of adrenaline for the non-α_2-adrenoceptor binding site (without GTP pK_i = 7.0±0.2; with GTP pK_i = 6.2±0.3, n=3) but did not alter the affinity of cirazoline.

	K_d (nM)	B_{max} (fm/mg protein)
^{3}H-Idazoxan	0.70±0.16	120±6
^{3}H-Idazoxan+Yoh.	1.23±0.17	97±6
^{3}H-Idazoxan+GTP	0.96±0.03	89±1
^{3}H-Idazoxan+Yoh+GTP	1.37±0.27	71±6

The data provide preliminary evidence that the idazoxan binds to an α_2-adrenoceptor and a non-α_2-adrenoceptor binding site in bovine cerebral cortex. The latter site may represent receptors linked with GTP binding proteins and both idazoxan and adrenaline might be agonists at these receptors.

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270P EFFECTS OF HISTAMINERGIC MODULATION ON BODY TEMPERATURE IN MICE

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Evidence suggests that histamine has physiological roles in the mammalian hypothalamus, including temperature regulation. From results obtained where histamine and histamine agonists and antagonists were administered intracerebrally in cats and rats, both H_1 and H_2 receptors were reported to be involved (Green, Cox & Lomax, 1976). The recent development of centrally-penetrating H_2 antagonists facilitates investigation into central roles of histamine. The present studies were undertaken to investigate the effects of H_2 antagonists and of enhancing histamine levels on body temperature in mice. Groups of TO mice (25-30g) were pre-treated with metoprine, an inhibitor of histamine N-methyl transferase (Duch, Bowers & Nichol, 1978) (20 mg kg^{-1}) or l- or d-histidine (3 x 500-1000 mg kg^{-1} at 30 min intervals) and a histamine H_2 antagonist or appropriate vehicle administered intraperitoneally. In initial experiments, body temperatures were measured at 30 min intervals by rectal probe (Digimed, Linton Instrumentation). In later experiments mice with implanted thermistors (Minimitter Inc., USA) were used with a telemetry system developed at the Polytechnic of East London (Jakobson & Andrews, 1989) to obtain continuous recordings of body temperature from undisturbed animals. All experiments were carried out at an ambient temperature of 19-22°C. Statistical analyses were carried out using a Mann Whitney U test or one-way ANOVA. Metoprine alone produced a significant ($p < 0.01$) hypothermia of -2.9°C with a maximal effect at 30 min after administration (n=5). Similarly l-histidine caused dose-dependent hypothermia with the maximal effect (-4.0°C ($p < 0.001$)) within 1h after the last precursor loading dose (n=30). D-histidine had less effect on the body temperature. Ranitidine (20-50 mg kg^{-1}), a non-cns penetrating H_2 antagonist, failed to modify l-histidine-induced hypothermia, while cimetidine (5-12 mg kg^{-1}) and zolantidine (2-8 mg kg^{-1}) (H_2 antagonists which penetrate the cns to varying degrees) antagonised l-histidine-induced hypothermia ($p < 0.001$) (n=30). The H_2 antagonists alone were without significant effect on the body temperature. Thus increasing histamine levels by either precursor loading or metabolic inhibition induces hypothermia in mice and these effects can be antagonised by centrally- but not peripherally-acting histamine H_2 antagonists.

We wish to thank Smith Kline Beecham for the gift of zolantidine, Glaxo Group Research for ranitidine and Burroughs Wellcome for metoprine.

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271P A PHYSIOLOGICAL MODEL OF FENTANYL PHARMACOKINETICS FOR VARIOUS ROUTES OF ADMINISTRATION

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We present a physiological model for the simulation of fentanyl distribution and elimination, based on Mapleson's model of the kinetics of inhaled anaesthetic agents, Model P following the procedure used by Davis (Davis & Mapleson, 1981; Davis, 1987) in his adaptation for injected pethidine. We have added a nasal compartment to allow the simulation of nasally administered drug.

The model comprises a representation of the blood circulation around an idealised man whose bloodless body mass is divided into discrete compartments on the basis of anatomy and perfusion characteristics. Blood is circulated in discrete aliquots. Both the rates of blood volume flow and the mean transit time through each compartment are represented. At each calculation cycle, perfusion-limited drug distribution between each of the tissue compartments and the corresponding circulating blood is modelled by calculating the equilibrium distribution of drug between erythrocytes and plasma and of ionised and unionised drug species between the water, fat and protein in the plasma, tissue cells and extracellular fluid. Metabolism is modelled by removing a set fraction of drug from blood perfusing the liver and excretion by including an extra aqueous compartment into which fentanyl is concentrated when calculating distribution in the kidney block. Keeping a tally of drug movement allows a realistic representation of drug elimination and of distribution between the tissues and different regions of the circulation at any time.

The model was quantified for fentanyl using existing published data. Where experimental values are not available, as for solubility in body lipids for example, parameters have been estimated (Higgins, 1991). Protein binding has been characterised using data on plasma protein binding.

The model has been evaluated retrospectively against existing studies of fentanyl pharmacokinetics (Higgins, 1991). This preliminary assessment suggests that simulated arterial and venous plasma concentrations are similar to those obtained experimentally though we note the fairly wide range of disagreement between these studies. There is some evidence that the realism of the model would be improved by increasing the distribution of fentanyl to the lungs.

The model exists as a computer program in Turbo Basic suitable for use on an IBM-compatible micro-computer. Drug administration may be intramuscular, intravenous, intranasal or intrapulmonary. The model allows simulation of changes in cardiac output, plasma pH, urine output and urine pH. Output comprises cumulative amounts of drug administered, metabolised and excreted and amounts and concentrations of drug in various tissue compartments and in the circulation. The model will be demonstrated at the meeting.

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272P A NOVEL METHOD OF MEASURING THE [Ca^{2+}] WITHIN PERMEABILIZED SMOOTH AND CARDIAC MUSCLE PREPARATIONS

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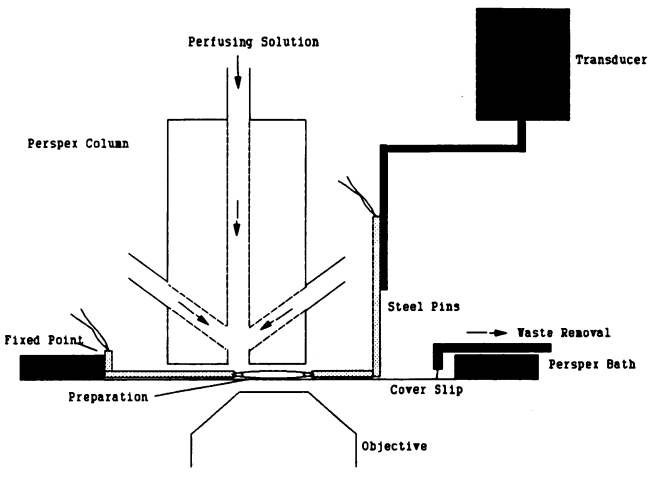

Permeabilized muscle preparations have been widely used to study the sensitivity of contractile proteins to Ca^{2+}. Recent permeabilizing treatments have preserved sarcoplasmic reticulum function and permitted the study of Ca-release and uptake processes. We have developed a technique to measure [Ca^{2+}] within these preparations using the fluorescent dye Indo-1 while simultaneously monitoring the tension. As shown in the figure, the permeabilized muscle is attached between a fixed support and a tension transducer in a shallow bath. The bath is placed on a Nikon Diaphot inverted microscope. A perspex column is lowered onto the muscle to minimise the volume of the solution above and below the preparation. The perfusing solution containing Indo-1 (4μM) is pumped down the centre of the column at a rate of 1ml/min. The solution around the muscle can be rapidly changed using the manifold at the base of the column. By illuminating the preparation with light of wavelength 465nm, the average [Ca^{2+}] within the preparation can be monitored by measuring the ratio of the emitted light intensities at 405nm and 495nm. Light emitted from areas of the visual field not occupied by the image of the muscle is reduced using a variable rectangular window on the side camera port before the TV camera and photomultiplier tubes.

273P METHOHEXITONE INHIBITION OF K^+-STIMULATED RELEASE OF [^{3}H]NORADRENALINE FROM MINI-SLICES OF RAT CEREBRAL CORTEX IS DEPENDENT ON K^+ CONCENTRATION

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Previous experiments have shown high concentrations of barbiturate anaesthetics to cause a dose-dependent inhibition of K^+-stimulated release of [^{3}H]noradrenaline from minislices of rat cerebral cortex (Jones & Symington, 1990). The present study investigated whether the response to methohexitone would depend on the K^+ stimulus used.

Minislices (350 μm x 350 μm) of rat cerebral cortex loaded with [^{3}H]noradrenaline were superfused with a modified Krebs-phosphate solution containing pargyline (10^{-5} M) and desipramine (10^{-7} M) at 500 μl min^{-1}. After equilibration fractions of superfusate were collected at 2 min intervals. Release of [^{3}H]noradrenaline was stimulated by 2 min pulses of high K^+ Krebs solution introduced at 6 and 30 min. Methohexitone was introduced at 18 min. [^{3}H]Noradrenaline efflux was estimated by liquid scintillation counting. Results were calculated as the S_2/S_1 ratio (where S_2 and S_1 are the increase in release over basal observed during the 10 min period after each K^+ stimulus, expressed as a fraction of the total tissue content of [^{3}H]noradrenaline). These are summarised in Table 1 as percentage of the S_2/S_1 ratio for methohexitone-free controls.

Table 1 Effect of K^+ concentration on response to methohexitone

Methohexitone concentration (M):	10^{-5}	3 x 10^{-5}	10^{-4}
12.5 mM K^+	94 ± 9	88 ± 9	**52 ± 8
25 mM K^+	93 ± 5	**72 ± 4	**42 ± 4
50 mM K^+	*70 ± 5	*69 ± 5	**42 ± 6

Results are % control, mean ± s.e. mean, n = 7-27, *$p<0.01$, **$p<0.001$, Students t test.

The response to a given concentration of methohexitone appears to be larger with 50 mM K^+ stimulation than with 12.5 mM K^+. The lowest concentration of methohexitone observed to cause a significant reduction in [^{3}H]noradrenaline release was 10^{-5} M for 50 mM K^+, 3 x 10^{-5} M for 25 mM K^+ and 10^{-4} M for 12.5 mM K^+. These results suggest that the variability in responses to methohexitone previously reported may be due, at least in part, to differences in the exposure to K^+ of the brain tissue. Barbiturate anaesthetics are thought to inhibit neurotransmitter release by decreasing Ca^{++} entry into presynaptic terminals in response to depolarisation. Other barbiturate actions, such as an increased Cl^- conductance, which alter the membrane potential may also affect neurotransmitter release. The degree of depolarisation (determined by K^+ concentration) will determine the exact population of voltage-dependent ion channels functioning, and those populations may show differential sensitivity to methohexitone. Alternatively, different K^+ concentrations may release noradrenaline from different intracellular pools, which may respond differently to methohexitone. The mechanism remains to be determined.

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274P EFFECT OF TIZANIDINE ON SYNAPTIC ACTIVITY OF RAT *IN VITRO* SPINAL CORD PREPARATIONS

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The centrally acting muscle relaxant drug tizanidine has been reported previously to have no depressant action on in vitro amphibian spinal preparations (Coward et al, 1984). In vivo studies in the rat spinal cord have revealed α_2-adrenoceptor agonist activity of tizanidine (Davies and Quinlan, 1985). Kendig et al (1991) showed that long duration components of the dorsal root evoked ventral root potential (DR-VRP) of in vitro immature rat spinal preparations were depressed by the α_2-adrenoceptor agonist clonidine. Thus we have examined the action of tizanidine on the DR-VRP of hemisected spinal cord preparations from the immature rat to show whether this preparation could be useful as a screen for this type of myorelaxant drug. The preparations were superfused with medium containing 1.25 mM $MgSO_4$ and 1.5 mM $CaCl_2$ at 25°C. In the presence of tetrodotoxin (0.1 µM) depolarizations induced by NMDA (10 to 20 µM) were unaffected by tizanidine (10 to 100 µM). Supramaximal electrical stimulation (0.011 Hz) of a dorsal root evoked DR-VRPs with a monosynaptic peak amplitude of 6.40 mV ± 1.49 s.e.mean and an area (aVRP), measured between 2 and 32 seconds of 15.2 ±2.4 mV.sec (N=4). Application of tizanidine (0.03 - 3 µM) for 30 to 45 min depressed aVRP to 23.8 ±1.4% of control values (EC_{50} 63.8 ±11.4 nM, N=4). In two preparations the tizanidine-induced depression of aVRP was not reversed by the α_1-adrenoceptor antagonist prazosin (0.5 µM) or the opioid antagonist naloxone (1 µM). However, the depressant action of tizanidine was reversed by subsequent application of idazoxan (1 µM) to these preparations (dose ratio 115 ±28, N=3).

The monosynaptic population spike of the DR-VRP was found to be resistant to the depressant action of tizanidine. In order to test the tizanidine-sensitivity of the underlying monosynaptic population e.p.s.p., sufficient kynurenate was applied to block the population spike. In the presence of 1 mM kynurenate the population e.p.s.p. had a mean latency of 6.3 ±0.9 msec. Under these conditions the rising slope of the e.p.s.p. (0.086 ±0.031 mV per msec) was not significantly altered by tizanidine (1 µM) (0.087 ±0.030 mV per msec). It is possible therefore that the α_2-adrenoceptors, at which the depressant effect of tizanidine is mediated, are not present on spinal primary afferent terminals.

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275P BRAIN α_2-ADRENOCEPTOR BINDING SITES IN ANTIDEPRESSANT-TREATED SUICIDES AND CONTROLS

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We have previously compared α_2-adrenoceptor binding sites (by saturation binding of [^{3}H]rauwolscine) in cortical samples from depressed suicides, who had not recently received antidepressant drugs, and matched controls. The number of sites was higher in frontal (by 10%) and occipital (by 13%) cortex and significantly so (by 24%) in temporal cortex (Ba 21/22) of suicides compared to controls (De Paermentier et al, 1991a). To provide an indication of the effects of antidepressant treatment we have extended the study to depressed suicides (8M, 9F, mean age ± sem 44±3 years, post-mortem delay 39±4 hr) who had been received antidepressant drugs (alone or in combination with other drugs) and matched controls (8M, 8F, 45 ±4 years, 41±4 h). Bmax values (mean ± SEM) with the percentage differences between antidepressant-treated (AT) suicides and controls, antidepressant free (AF) suicides and controls (from De Paermentier et al, 1991a) and the effect of antidepressant treatment (difference between AF and AT) are shown in Table 1.

Table 1. [^{3}H]Rauwolscine Bmax (fmol /mg protein) in antidepressant-treated suicides and controls

	α_2-NA Bmax		% change	% change	Effect of
Cortical region	Controls	Suicides	AT	AF	Treatment
Frontal Ba 11	244 ± 10	269 ± 38	+ 10	+ 11	+ 1
Occipital Ba 17/18	257 ± 14	205 ± 13*	- 21	+ 13	- 34
Temporal Ba 21/22	238 ± 11	234 ± 25	- 2	+ 24	- 26
Temporal Ba 38	258 ± 13	240 ± 10	- 7	- 4	- 3

Bmax values for antidepressant-treated suicides did not differ from matched controls in frontal or temporal cortex (Ba 38 and Ba 21/22) but were significantly lower in occipital cortex of suicides (by 21%, $p<0.05$). The overall effect of antidepressant treatment on α_2-adrenoceptor binding appeared to differ between cortical areas. In occipital and temporal (Ba 21/22) cortex, treatment reduced Bmax by 26-33% but had no effect in frontal or temporal (Ba 38) cortex. These findings contrast with the general lack of effect of antidepressant treatment on cortical β adrenoceptor binding in similar groups of subjects (De Paermentier et al, 1991b).

We thank the Sir Jules Thorn Charitable Trust for financial support.

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276P PHOSPHATIDYLINOSITOL POTENTIATES THE EFFECT OF AMITRIPTYLINE ON β_1-ADRENOCEPTOR NUMBER AND ON NORADRENALINE-STIMULATED ADENYLYL CYCLASE ACTIVITY IN RAT FRONTAL CORTEX

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Administration of phospholipid liposomes is reported to potentiate the effects of antidepressant drugs in the behavioural despair test (Drago et al, 1985). In vitro the stimulation of adenylyl cyclase by isoprenaline is inhibited by addition of acidic phospholipids (Kirilovsky & Schramm, 1983). We have therefore investigated the effect of administration of an individual acidic phospholipid, phosphatidylinositol (PI), on the action of amitriptyline (AMI) on ß-adrenoceptor binding and activation of adenylyl cyclase in rat frontal cortex.

Male Wistar rats were treated with either AMI (10mg/kg i.p.) or saline and either PI liposomes (15mg/kg i.p.) or vehicle daily for 4 or 14 days. Control animals received saline and vehicle. Animals were killed 24h after the last injection and the frontal cortex immediately removed and stored at -20°C until assay. β_1-Adrenoceptor number and affinity of membrane homogenates were characterised using ^{125}I-pindolol displaced by 100nM CGP 20712A. Adenylyl cyclase activity was measured in slices of rat frontal cortex by conversion of ^{3}H-adenine to ^{3}H-cAMP on stimulation with noradrenaline (10 - 300 µM).

Table 1: Binding capacity (Bmax; fmol/mg protein) of β_1-adrenoceptor and stimulation of adenylyl cyclase (AC) by noradrenaline (300µM) following treatment with AMI and/or PI (basal response = 100%). Results are expressed as mean ± s.e.m.
Statistical comparisons by Newman-Keuls test within 2-way ANOVA: * $p<0.05$; ** $p<0.01$ v CONTROL ; † $p<0.05$ v AMI

	n	4 DAYS Bmax	14 DAYS Bmax	n	4 DAYS % Stimulation AC
CONTROL	8	76 ± 2	81 ± 2	6	206 ± 19
AMI	8	81 ± 3	73 ± 2 *	8	128 ± 11 **
PI	4	72 ± 3	80 ± 3	4	205 ± 26
AMI + PI	4	69 ± 3 †	67 ± 2 **	4	100 ± 10 **

After 4 days treatment with AMI or PI alone there was no change in β_1-adrenoceptor number. However when PI was administered with AMI there was a significant down-regulation of β_1-adrenoceptors compared to administration of AMI alone. Following 14 days treatment, receptor number was significantly reduced by AMI both with and without PI. There were no changes in binding affinity. Stimulation of adenylyl cyclase by noradrenaline was reduced by 38% after 4 days treatment with AMI and by 51% following AMI + PI.

We conclude that co-administration of phosphatidylinositol and amitriptyline may enhance the rate of β_1-adrenoceptor down-regulation and the reduction in adenylyl cyclase activity relative to the action of amitriptyline alone. Further studies are planned to investigate the relevance of this interaction to other antidepressant treatments.

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277P THE EFFECT OF THIOPERAMIDE ON *EX VIVO* [^{3}H](R)-α-METHYLHISTAMINE BINDING TO RAT BRAIN HISTAMINE H_3 RECEPTORS

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The selective histamine H_3 receptor agonist [^{3}H](R)-α-methylhistamine (RAMH) has been shown to label H_3 receptors in homogenates of rat cortex (Arrang et al., 1987; Kilpatrick and Michel., 1991). We have used an *ex vivo* binding approach to examine the effect of prior administration of the selective H_3 receptor antagonist thioperamide on [^{3}H]RAMH binding in homogenates of the rat brain cortices.

Thioperamide (0.02-5 mg/kg, 2ml/kg, i.p.) or thioperamide vehicle (0.9% NaCl) was administered to male Lister Hooded rats (250-300g). At various times later (0-8 hrs) the animals were anaesthetized using isoflurane and perfused transcardially with ice-cold HEPES buffer (20ml, 50mM, pH 7.4). Cerebral cortices from individual animals were dissected and homogenised (Ultra-Turrax) in an identical HEPES buffer (7.5ml/g tissue). Binding of [^{3}H]RAMH was assessed as described previously (Kilpatrick and Michel, 1991) except that the tissue washing stage was omitted. In animals dosed with thioperamide, results were expressed as a percentage inhibition of the specific [^{3}H]RAMH binding in animals dosed with thioperamide vehicle (where specific binding was assigned to be 100%).

In vehicle dosed rats, the specific [^{3}H]RAMH binding remained constant (total and non-specific binding = 748±39 and 119±5 dpm respectively; mean±s.e.mean, n=20) throughout the experimental time course used in this study. However, pretreatment of rats with thioperamide markedly inhibited specific [^{3}H]RAMH binding. The threshold dose for significant inhibition of binding was 0.2 mg/kg (20±5%, mean±s.e.mean, n=4). At doses of 1.0 mg/kg and above, peak inhibition of binding was observed 30 mins after dosing. At this time the apparent ED_{50} for thioperamide was approximately 1.5 mg/kg. At doses of 2 and 5 mg/kg significant inhibition of binding was evident for at least four hours. It is possible that the threshold dose and ED_{50} value quoted above are over-estimates because it was necessary to dilute the tissue 75 fold in order to perform the binding assay. We have not attempted to correct the results to take this into account because the thioperamide dilution factor may be less than this due to retention of thioperamide by the H_3 receptor.

In view of the limited *in vivo* data on H_3 receptor compounds, we conclude that *ex vivo* [^{3}H]RAMH binding could be a useful technique for estimating the central penetration and time course of agents which interact with the H_3 receptor.

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278P IRREVERSIBLE BLOCKADE OF PERIPHERAL AND CENTRAL HISTAMINE H_3 RECEPTORS BY N-ETHOXYCARBONYL-2-ETHOXY-1,2-DIHYDROQUINOLINE

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N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) has been reported to act as an irreversible antagonist at various receptors (Belleau et al., 1968). We now report on the effects of EEDQ at histamine H_3 receptors in the periphery and brain using two in vitro assays of H_3 receptor activity: binding of the selective H_3 receptor agonist [^{3}H](R)-α-methylhistamine ([^{3}H]RAMH) to homogenates of rat brain cortex (Arrang et al., 1987) and the guinea-pig isolated ileum longitudinal muscle-myenteric plexus preparation (Taylor et al., 1991).

Binding of [^{3}H]RAMH (0.02-10nM) to H_3 receptors in rat cortex homogenates was performed as described previously (Kilpatrick and Michel, 1991), except that prior to addition of the ligand the tissue homogenates were incubated in the presence of EEDQ (3×10^{-6}M) or EEDQ vehicle for 30 minutes at 37°C and then washed twice by centrifugation. In vehicle treated tissue, saturation analysis revealed a single saturable site (K_D=0.84±0.14 nM; B_{max}=105±9 fmol/mg protein; mean±sem, n=3). However, incubation of tissue homogenates with EEDQ resulted in a decrease in the apparent number of binding sites (B_{max}=63±9 fmol/mg protein; n=3) with no effect on the affinity of [^{3}H]RAMH for its binding site (K_D=0.74±0.12 nM; n=3).

In the field stimulated guinea-pig ileum we have determined the effect of EEDQ on the inhibitory responses elicited by the H_3 receptor agonists RAMH, N^{α}-methylhistamine (NAMH), N^{α},N^{α}-dimethylhistamine (NADMH) and histamine. For each agonist a concentration-effect curve was produced, the tissues washed and contractions allowed to recover as described previously (Taylor et al., 1991). Exposure of the tissues to EEDQ (10^{-6}M) for 15 or 30 minutes, followed by extensive washing had no effect on basal contractions but resulted in a non-parallel rightward shift of each agonist concentration-effect curve with a decrease in the maximum inhibitory effect obtainable. An irreversible antagonist can be used to estimate agonist affinity values and we have used the null method of Furchgott (1966) to estimate the following apparent affinity (pK_D) values for the H_3 receptor agonists used in this study: RAMH 7.01±0.05; NAMH 7.06±0.17; NADMH 6.09±0.26; histamine 6.13±0.10 (mean±sem, n≥4).

From the data presented in this study, we conclude that EEDQ acts as an irreversible antagonist at H_3 receptors in both the periphery and brain. As such, we believe this compound should provide a useful tool for further investigation of the H_3 receptor.

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279P CHLORMETHIAZOLE IS NEUROPROTECTIVE FOLLOWING TRANSIENT FOREBRAIN ISCHAEMIA WHEN GIVEN AS AN INTRAVENOUS INFUSION TO THE GERBIL

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Chlormethiazole (CMZ) administered intraperitoneally (i.p.) at a dose of 620μmolkg^{-1} prevents delayed neurodegeneration in gerbil hippocampus following transient forebrain ischaemia (Cross et al, 1991). We have now devised a procedure for intravenous administration of the drug which produces either a similar peak plasma concentration to that seen after CMZ (620 μmolkg^{-1} i.p.) or half that value.

Male Mongolian gerbils (60-80g) were anaesthetised with alphaxalone (45mgkg^{-1} i.p.) and carotid arteries occluded for 5 min. CMZ was given 30 min later either i.p. (620μmolkg^{-1}) or i.v. (high dose: 53μmolkg^{-1}min^{-1} for 10 min then 20μmolkg^{-1}min^{-1} for 20 min; low dose: 26.5μmolkg^{-1}min^{-1} for 10 min then 10μmolkg^{-1}min^{-1} for 20 min). Rectal temperature was maintained at 37°C. The damage to CA1 region (as a % of the whole CA1 region) was assessed in cresyl violet stained sections. In a separate experiment, plasma CMZ concentrations were measured using h.p.l.c. with UV detection (Kim & Khanna, 1983).

Table

Group	Plasma CMZ conc (nmolml^{-1}) t=10	t=30	%Damage CA1 region
i.v. Saline	0	0	81.7 ± 8.1
i.v. CMZ low dose	-	75.3 ± 6.7	53.4 ± 13.7**
i.v. CMZ high dose	202.5 ± 52.4	228.5 ± 9.2	16.8 ± 5.6**
i.p. Saline	0	0	78.1 ± 12.0
i.p. CMZ	185.0 ± 26.0	86.0 ± 9.0	26.2 ± 13.0**

Mean + s.e.mean. (n=6-12). Different from saline: ** p<0.01 (ANOVA). t=time after start of drug administration (min).

These data indicate that when CMZ is given as an intravenous infusion with peak plasma concentrations similar to those seen after i.p. injection it is a very effective neuroprotective agent. The drug is also neuroprotective when infused at half that dose (Table).

To be used successfully in the treatment of stroke or cardiac arrest a neuroprotective agent is likely to have to be given intravenously. These data demonstrate that chlormethiazole given via this route in gerbils is neuroprotective.

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280P CHLORMETHIAZOLE PREVENTS METHAMPHETAMINE-INDUCED NEUROTOXICITY: STUDIES ON RAT BRAIN 5-HT AND DOPAMINE CONCENTRATIONS

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Repeated administration of high doses of methamphetamine (METH) produces neurotoxicity and decreased concentrations of 5-HT and dopamine in several regions of rat brain (Koda & Gibb, 1973; Hotchkiss & Gibb, 1980). Dizocilpine protects rats from the striatal dopamine loss (Sonsalla et al, 1991) and is a neuroprotective agent in the gerbil model of transient focal ischaemia (Gill et al, 1988). Since chlormethiazole (CMZ) is also an effective neuroprotective agent in the gerbil (Cross et al, 1991) we have studied the effect of CMZ in methamphetamine-induced neurotoxicity.

Rats were injected with CMZ (50mgkg^{-1}) or saline followed 15 min later by METH (15mgkg^{-1}). This procedure was repeated 3 times at 3h intervals. Three days later rats were killed, brain regions dissected out and striatal dopamine and cortical 5-HT measured by h.p.l.c. with electrochemical detection.

Repeated METH produced around a 70% loss of dopamine or 5-HT in the areas examined (Table). This loss was prevented by administration of CMZ before each injection of METH (Table).

The mechanism of METH induced neurotoxicity is unknown. However it can be prevented by several competitive and non-competitive NMDA antagonists (Sonsalla et al, 1991). The present study has demonstrated that chlormethiazole is also an effective neuroprotective agent, although CMZ does not alter [^{3}H]-MK 801 binding (Cross et al, unpublished).

Table Effect of chlormethiazole on methamphetamine induced neurotoxicity

Treatment	Striatal dopamine	Cortical 5-HT
Saline	5460 ± 210 (12)	320 ± 28 (13)
METH	1380 ± 388 (6)*	85 ± 18 (7)*
METH/CMZ	5840 ± 588 (16)	365 ± 36 (10)
CMZ	6430 ± 390 (12)	341 ± 25 (17)

All amine concentrations reported in ng g^{-1} (wet weight tissue) expressed as mean ± s.e.mean, (n).
*Different from other groups: p<0.001.

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281P DECREASE IN NIGRAL REDUCED GLUTATHIONE LEVELS IN PARKINSON'S DISEASE

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Previously, Perry and colleagues (1986,1988) reported a decrease in the levels of reduced glutathione in the substantia nigra (SN) of patients dying with Parkinson's disease. Using a different technique Slivka and colleagues (1987) criticised the findings on the grounds that the values of oxidised and reduced glutathione (GSH:GSSG) for control patients did not reflect the oxidative state of the brain, but they did not themselves study Parkinson's disease. We have now used the techniques employed by Slivka and colleagues to measure the levels of GSSG and GSH in brain areas from control subjects, patients with incidental Lewy body disease (preclinical Parkinson's disease) and idiopathic Parkinson's disease.

Brain tissue was obtained after autopsy from normal control patients with a mean age (68.3 $\pm$ 5.7 years), time between death and removal of brain (14.2 $\pm$ 1.5 h); Lewy body positive control subjects with a mean age (68.2 $\pm$ 5.8 years), time between death and removal of brain (21.3 $\pm$ 2.2 h) and Parkinson's disease patients with a mean age (79.2 $\pm$ 2.3 years), time between death and removal of brain (11.0 $\pm$ 3.1 h). Brain tissue was homogenised in 0.4 M perchloric acid and centrifuged, the supernatant was analysed for GSSG by an enzymatic recycling procedure using a double-beam spectrophotometer and GSH using high pressure liquid chromatography with ultraviolet detection.

The ratio of GSH:GSSG was high in all brain areas in normal control patients. There was no decrease in the levels of GSSG or GSH in the cortex (Brodmann area 10) or globus pallidus from Lewy body positive patients or patients with Parkinson's disease compared to control tissues. However, in the SN there was a decrease in the levels of GSH in both Lewy body positive patients and patients with Parkinson's disease (Table 1).

Table 1. Glutathione contents in normal controls, Lewy body positive and parkinsonian patients

Brain region	Glutathione content (μmoles/g wet weight) GSH (reduced)	GSSG (oxidised)
Substantia nigra		
Normal controls	0.80 $\pm$ 0.03 (3)	0.0027 $\pm$ 0.0004 (8)
Lewy body positive	0.51 $\pm$ 0.06* (6)	0.0026 $\pm$ 0.0006 (4)
Parkinson's disease	0.47 $\pm$ 0.06**(6)	0.0053 $\pm$ 0.0009* (3)

Values are mean $\pm$ SEM. Number of patients in parentheses. * $p < 0.05$, ** $p < 0.01$ as compared to normal controls (ANOVA).

The results confirm the earlier findings of Perry and colleagues that nigral GSH levels are decreased in Parkinson's disease, supporting the concept of oxidative stress as a cause of this illness. The reduction of GSH in the SN of preclinical patients suggests the occurrence of a toxic process in the early stages of the disease which involves the glutathione system.

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282P AUTORADIOGRAPHIC STUDY OF STRIATAL D_1 AND D_2 DOPAMINE RECEPTORS IN 6-OHDA-LESIONED RATS RECEIVING FOETAL NIGRAL GRAFTS AND L-DOPA TREATMENT

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Implantation of foetal dopamine cell grafts reverses the increase in D-2 dopamine receptor density produced by a unilateral 6-hydroxydopamine (6-OHDA) lesion in rats (Freed et al 1983), but the effect on D-1 receptors is unknown. Also, the effect of concurrent treatment with L-DOPA on D-1 and D-2 receptor densities has not been investigated. We now report the effects of foetal dopamine cell grafts on the regional density of D-1 and D-2 dopamine receptors, using 3H-SCH 23390 (D-1) and 3H-spiperone (D-2) autoradiography, in rats with a unilateral 6-OHDA lesion of the medial forebrain bundle, treated with L-DOPA and carbidopa. Foetal dopamine cells (FG) or sham grafts (SG) were implanted into the 6-OHDA lesioned striatum. Some animals were then treated with L-DOPA (200mg/kg/24h) and carbidopa (25mg/kg/24h) for 5 weeks, in the drinking water. Foetal grafts abolished (+)-amphetamine (5mg/kg ip)-induced ipsilateral rotation, and reduced apomorphine (0.5mg/kg sc)-induced contralateral rotation. by 40-50% compared to sham grafted rats. Specific binding for each ligand was obtained from autoradiograms which were analysed using computerised densitometry (IBAS 2000, Kontron), over the right and left striata in each of 4 quadrants at 3 A-P levels. A 6-OHDA lesion increased specific 3H-spiperone binding in the lateral and dorsomedial quadrants of the striatum (Table 1). Foetal grafts implanted into the lesioned striatum restored specific 3H-spiperone binding to levels found in the intact striatum. L-DOPA treatment did not alter the lesion-induced changes, or the effect of foetal grafts on specific 3H-spiperone binding. A 6-OHDA lesion caused a decrease in specific 3H-SCH 23390 binding of 18% in the ventrolateral quadrant, and a decrease of 16% in the dorsolateral quadrants, of the mid-body of the lesioned striatum. Neither a foetal graft nor L-DOPA treatment, nor a combination of the two altered the lesion-induced changes in 3H-SCH 23390 binding. The effect of an imbalance between D-1 and D-2 receptor function under these circumstances remains unknown.

TABLE 1.Specific 3H-spiperone binding in striatal slices through the level of sham or foetal graft placements over the whole striatum and individual quadrants (mean values are shown in fmols/mg tissue)

	Whole			Dorsolateral			Ventrolateral			Dorsomedial		
	intact	lesion	%	intact	lesion	%	intact	lesion	%	Intact	lesion	%
lesion + SG (n=4)	50.3	*63.5	25.2	46.8	*59.0	26.1	64.	*79.3	22.0	51.4	*65.2	26.8
lesion + SG + + L-DOPA (n=4)	56.3	*67.7	21.1	52.3.	*78.2	28.1	67.8	*90.7	31.9	49.5	*66.5	33.3
lesion + FG (n=6)	59.0	57.0	-3.4	64.0	61.9	-2.5	75.2	72.5	-4.0	49.0	50.6	4.8
lesion + FG + L-DOPA (n=6)	61.8	57.4	-7.2	66.2	58.3	-6.3	75.3	68.4	-9.0	58.8	52.0	-8.2

* $p<0.05$ compared with intact side, Student's t-test.

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283P AUTORADIOGRAPHIC STUDY OF D_1 AND D_2 DOPAMINE RECEPTOR DENSITIES IN THE BASAL GANGLIA OF THE MPTP-TREATED COMMON MARMOSET

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In MPTP-treated (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) primates there have been reports of increases, decreases or no change in striatal dopamine receptor density (Graham et al., 1990; Gagnon et al., 1990; Bedard et al., 1986). We now report on the changes in D-1 and D-2 receptor densities in the MPTP treated marmoset using [^{3}H]-SCH 23390 and [^{3}H]-spiperone respectively.

MPTP (total dose 6-13 mg/kg; sc) was administered to seven marmosets of either sex over 5-7 days. Nine untreated animals were used as controls. Survival times ranged between 13 to 792 days. The brains were removed and frozen in isopentane at -45°C. Brain sections were incubated with 0.5 nM [^{3}H]-SCH 23390, 0.5 nM [^{3}H]-spiperone or 4 nM [^{3}H]-mazindol. Specific binding was defined using 10 µM cis-flupentixol, 10 µM sulpiride and 10 µM mazindol for D-1, D-2 and dopamine uptake sites respectively. Regional densitometric analysis of autoradiographs revealed widespread depletion in [^{3}H]-mazindol binding in the striatum (caudate and putamen; 87-97%), nucleus accumbens (Acc; 30%) and substantia nigra pars compacta (SNc; 93%) of the MPTP-treated animals (Table 1). [^{3}H]-SCH 23390 binding was unaltered in the substantia nigra pars reticulata, medial segment of the globus pallidus and prefrontal cortex, but it was increased in the striatum (17-36%). Levels of [^{3}H]-spiperone binding were unaffected except in the SNc where it was reduced by 49% (Table 1).

Table 1: Effect of MPTP on D-1, D-2 dopamine receptors and dopamine uptake sites: specific binding in nCi/mg ± s.e.mean; Student's t-test (*P<0.001; **P<0.0001).

	[^{3}H]-SCH 23390		[^{3}H]-spiperone		[^{3}H]-mazindol	
	Control	MPTP	Control	MPTP	Control	MPTP
Putamen (dorsolateral)	10.9 ± 0.4	13.8 ± 0.6*	14.8 ± 0.4	15.2 ± 1.7	11.3 ± 0.4	0.9 ± 0.1**
Caudate (dorsolateral)	10.7 ± 0.3	14.0 ± 0.7**	15.4 ± 0.6	16.0 ± 1.9	10.3 ± 0.2	0.9 ± 0.3**
Acc	9.0 ± 0.4	9.7 ± 0.7	8.5 ± 0.7	6.7 ± 1.0	5.3 ± 0.2	3.8 ± 0.5**
SNc	---	---	2.9 ± 0.2	1.5 ± 0.3*	2.8 ± 0.3	0.2 ± 0.1**

This study further demonstrates denervation induced receptor supersensitivity in the striatum. The lack of change in striatal D-2 receptor binding may reflect differences in survival times, severity of lesion and/or response variation between species to MPTP.

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284P EFFECT OF α-METHYL-P-TYROSINE (AMPT) ON LOCOMOTOR ACTIVITY PRODUCED BY DOPAMINE AGONISTS IN MPTP-TREATED COMMON MARMOSETS

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In MPTP-treated primates the D-2 agonist quinpirole produces a reversal of motor deficits with resulting locomotor hyperactivity. However, the D-1 agonist SKF 38393 does not increase motor function and decreases the effects of quinpirole (Nomoto et al., 1988). This contrasts with the ability of SKF 38393 to potentiate the effects of D-2 receptor stimulation observed in rats. One explanation for this discrepancy is that SKF 38393 is a partial agonist and there may be sufficient endogenous dopamine present in the brain of MPTP treated primates to prevent the expression of agonist activity. We now report on the effect of α-methyl-p-tyrosine (AMPT) pretreatment on the changes in motor activity produced by quinpirole and/or SKF 38393 in common marmosets treated with MPTP six months previously.

AMPT methyl ester hydrochloride (150 mg/kg, sc) was administered 18 and 2h prior to quinpirole (0.3 mg/kg, ip) or SKF 38393 (5.0 mg/kg, ip). All drugs were dissolved in 0.9% sterile saline. Motility counts for each animal were measured in automated activity cages and the motor disability was scored by an observer.

Table 1 : Total locomotor activity (mean counts ± s.e.mean/240 min) with or without AMPT.

	Vehicle	SKF 38393	Quinpirole	SKF 38393 & Quinpirole
Control	3,319 ± 399	2,377 ± 411	13,554 ± 2,165	10,732 ± 3,040
AMPT	236 ± 135*	160 ± 49*	6,039 ± 1239*	5,006 ± 839

*P<0.05, compared to controls, Student's t-test.

The MPTP-treated common marmosets showed low basal motor activity which was lowered further by the prior administration of AMPT (Table 1). Administration of quinpirole increased motor activity in MPTP treated marmosets but the effect was reduced by pretreatment with AMPT. SKF 38393 had no effect on motor function in either MPTP-treated animals or those pretreated with AMPT.

SKF 38393 caused some reduction in motor activity in MPTP-treated animals but this effect was not observed following AMPT treatment. Removal of endogenous dopamine stores in MPTP-treated common marmosets using AMPT did not reveal agonist activity for SKF 38393. Rather the effect of quinpirole appeared dependent on remaining dopamine production in MPTP-treated animals. This may support the view of endogenous D-1 tone being necessary for the expression of D-2 activity as occurs in rodents.

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285P THE PHARMACOLOGY OF NOVEL NEUROTENSIN ANALOGUES ON NEURONES IN THE RAT SUBSTANTIA NIGRA PARS COMPACTA, *IN VITRO*

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We have previously reported structure activity studies showing that biological activity of neurotensin (NT) on substantia nigra neurones *in vitro* is contained within the C-terminal 8-13 fragment (Keegan *et al*, 1990). The neurotensin analogue, kinetensin (KT), was inactive on neurotensin sensitive substantia nigra neurones. In contrast to the other active neurotensin fragments, kinetensin contains two amino acid substitutions; His at position 9 and Phe at position 12. In the present study, we have compared the effects of NT to those of kinetensin, His^9NT8-13, Phe^{12}NT8-13, Orn^8NT8-13, Arg^5KT and Ile^{12}KT.

Extracellular recordings were made as previously described (Pinnock, 1985). NT excited 107/113 dopamine neurones on which it was tested. The ED50 for NT was 13.13±4.8nM (n=17). The relative order of potency of the fragments was NT > His^9NT = Ile^{12}KT = NT8-13 = Orn^8NT8-13 >>> Phe^{12}NT8-13 = Arg^5KT >> KT. the data for NT analogues is summarised in Table 1. The kinetensin analogues (e.g.Ile^{12}KT) were equipotent to the neurotensin substituted derivatives (e.g His^9NT).

Table 1.

DRUG	NT	His^9NT	NT8-13	Orn^8NT 8-13	Phe^{12}NT 8-13	KT
ED_{50}(nM)	13.13	74.73	74.51	106.0	608.14	I.A.
SEM	4.8	22.52	25.65	29	217	I.A.
n	17	6	4	3	4	8

These results confirm that the biological activity of NT on SNC neurones resides within the C-terminal 5 amino acids. Substitution with His^8 makes little difference to the potency of the peptides as NT agonists. This suggests that a positively charged substitution either at Arg^8 or Arg^9 will be sufficient to retain activity. In contrast, substitution of Ile^{12} with nonpolar hydrophobic Phe leads to almost complete loss of activity. Thus, not only is the Tyr^{11} important for activity (Jolicoeur *et al*, 1985) but Ile^{12} is also critical for biological activity. Since none of the inactive analogues depressed the response to 1μM NT, it appears that these amino acid substitutions result in loss of binding to the receptor rather than loss of agonist efficacy.

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Jolicoeur, F.B., Rioux, F. and St-Pierre, S., 1985, Neurotensin, in: Handbook of Neurochemistry, Vol.8 (A. Lajitha, ed.), pp 94-114, Plenum Press, New York.

286P THE PHARMACOLOGY OF DOPAMINE D_2 RECEPTORS IN RAT STRIATUM AND THE TRANSFECTED $D_{2(444)}$ IN CHO CELLS

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Since the recent cloning of multiple forms of dopamine D-2 receptors (Bunzow et al., 1988), it has become important to identify the functional receptor(s) present in the brain. Radioligand binding studies using the D-2 selective antagonist [^{3}H]-sulpiride have been carried out on the long form of the rat D-2 receptor transfected into CHO cells, and compared with similar data from rat striatal membranes.

Lysed membrane preparations of the CHO cells (Passage 5-20) and a P2 membrane preparation of rat striata were incubated in 20mM HEPES Krebs' buffer pH7.4 (+10μM pargyline) with 10 and 3nM [^{3}H]-sulpiride respectively at room temperature for 10 min. Non-specific binding was defined by 1μM (-) sulpiride. Incubations were terminated by 2 min on ice followed by filtration through GF/C filters and washed with 15ml ice cold buffer. To measure GTP shifts, all membranes were washed in 1mM EDTA then assayed as above, in the presence of 10mM EDTA and 100μM guanylyl imidodiphosphate. (Table)

Table 1	Cells: Kapp (nM)			Striata: Kapp (nM)		
Compound	-EDTA/GppNHp	+EDTA/GppNHp	GppNHp shift	-EDTA/GppNHp	+EDTA/GppNHp	GppNHp shift
(-)Sulpiride	4.1(3.7;4.7)	3.9(2.7;5.7)	0.95	2.1(1.7;2.6)	2.5(2.2;3.0)	1.2
(+)Sulpiride	110(97; 120)	72(65;79)	0.65	59(38;91)	65(59;73)	1.1
Eticlopride	0.13(0.10;0.16)	0.11(0.091;0.13)	0.85	0.065(0.045;0.095)	0.099(0.062;0.16)	1.5
Quinpirole	1200(800;1700)	4500(3900;5200)	3.8	300(300;310)	3400(3000;3700)	11
Dopamine	390(270;550)	1300(1200;1500)	3.3	140(120;180)	1500(1200;1800)	11
Apomorphine	44(37;53)	59(55;63)	1.3	13(10;16)	58(54;62)	4.5

Kapp: IC_{50} corrected for ligand occupancy as the geometric mean, numbers in parentheses are the low and high error values. Each value is the mean of at least 3 independent determinations carried out in duplicate.

Table 1 shows that the rank order of affinity of standard dopaminergic compounds between the rat D-2 receptor in CHO cells and in rat striatal membranes were similar, as was the GTP shift. However, the G-protein linkage in the cells appears to be of a lower magnitude, e.g. quinpirole only had a GTP shift of 3.8 in the cells compared to 11 in the striatal membranes.

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287P STIMULATION OF LOCOMOTOR ACTIVITY BY BLOCKADE OF SOMATO-DENDRITIC DOPAMINE D_2 AUTORECEPTORS IN THE RAT

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Using a change-over design, (–) or (+) sulpiride (0–5.0 μg, bilaterally) were administered intracerebrally into dopaminergic projection areas in the ventral striatum or into the somato-dendritic region in the ventral tegmental area, 10 min before the start of a 18 min observation period of locomotor activity in an open field arena. The results, as shown in **Figure 1**, are expressed as mean activity per min ± S.D., based on the performance of 4–7 animals per group. The sulpiride enantiomers both produced a suppression of locomotor activity after the local application into the ventral striatum. In the ventral tegmental area, however, (–) sulpiride administration resulted in an increased locomotion, whereas the (+) enantiomer produced no effect or, at the highest dose (5.0 μg), a suppression of the locomotor activity. The statistical evaluation was performed by means of a two-way ANOVA for repeated observations as follows: **Ventral striatum:** $F(1,7)=0.09$, n.s. (treatment); $F(3,21)=15.90$, $P<0.001$ (dose); $F(3,21)=1.55$, n.s. (treatment x dose). **Ventral tegmental area:** $F(1,10)=30.24$, $P<0.001$ (treatment); $F(3,30)=1.20$, n.s. (dose); $F(3,30)=3.37$, $P<0.05$ (treatment x dose). Paired t-test comparisons with the saline treatment condition are indicated in **Figure 1** (n.s. $P>0.05$, * $P<0.05$, ** $P<0.01$). The results demonstrate that a dopamine (DA) D-2 antagonist produces different functional effects depending on blockade of postsynaptic DA receptors in projection areas or a blockade of DA autoreceptors in the cell body region of the ventral tegmental area. It should be noted that (+) sulpiride produced a suppression of locomotion upon local application not only into the ventral striatum, but also into the ventral tegmental area. One interesting explanation for this unexpected finding would be partial DA receptor agonist properties of the (+) enantiomer. Such properties should result in receptor blockade in an area like the ventral striatum where the receptor sensitivity normally is low, as compared to the cell body region where the same compound could exert weak agonist actions. **In conclusion:** A selective blockade of DA D-2 somato-dendritic autoreceptors produces a stimulation of locomotor activity in the rat. In dopaminergic projection areas, a blockade of postsynaptic DA D-2 receptors will predominate, resulting in behavioural suppression.

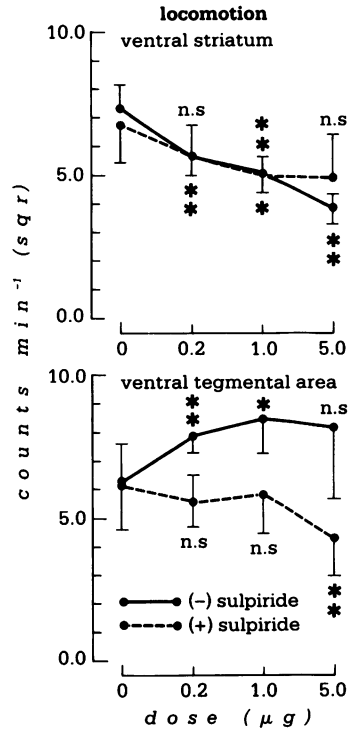

Figure 1. Effects of intracerebral administration of sulpiride enantiomers on locomotor activity in the rat.

288P PREJUNCTIONAL α_1-ADRENOCEPTORS IN THE RAT KIDNEY

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Recent studies have suggested that inhibitory prejunctional α_1-adrenoceptors exist in some tissues. The present study examined the nature and mechanisms of action of the inhibitory effect of the α_1-adrenoceptor agonist methoxamine on noradrenaline release from slices of rat kidney cortex. Slices of rat kidney cortex were incubated with [^{3}H]noradrenaline and then placed in a flow cell. The kidney slices were electrically field stimulated (5 or 1 Hz, 100 mA, 0.1 ms, 60 s) and the stimulation-induced outflow of radioactivity was taken as an index of noradrenaline release. At a stimulation frequency of 1 Hz, both methoxamine (10 μM) and the α_2-adrenoceptor agonist clonidine (0.1 μM) inhibited noradrenaline release from the kidney slices. The inhibitory effect of methoxamine was prevented by the α_1-adrenoceptor antagonist prazosin (0.1 μM), but not by the α_2-adrenoceptor antagonist idazoxan (0.1 μM). The inhibitory effect of clonidine was abolished by idazoxan, but was unaffected by prazosin.

The role of arachidonic acid metabolites and adenosine in the inhibitory effect of methoxamine on noradrenaline release was examined. Neither the prostaglandin synthesis inhibitor indomethacin (10 μM) nor the adenosine receptor antagonist 8-phenyltheophylline (1 μM) altered the inhibitory effect of methoxamine. However, the inhibitory effect of methoxamine was abolished by the arachidonate 5-lipoxygenase inhibitor BW A4C (1 μM).

Pertussis toxin was used to examine the role of G-proteins in the inhibitory effect of methoxamine. Methoxamine did not inhibit noradrenaline release from slices of rat kidney cortex taken from rats treated with pertussis toxin (25 μg/kg i.v., 4 days prior to experiment). However the inhibitory effect of clonidine was unaffected by the pertussis toxin treatment.

Taken together, these results suggest that the sympathetic nerves of the rat kidney possess prejunctional α_1- and α_2-adrenoceptors, which function independently to inhibit noradrenaline release. The prejunctional α_1-adrenoceptors function by stimulating 5-lipoxygenase through a pertussis toxin-sensitive G-protein. Prejunctional α_2-adrenoceptors are not coupled to a mechanism mediated by a pertussis toxin-sensitive G-protein.

289P ROLE OF CYCLIC AMP IN THE STIMULATION-EVOKED RELEASE OF NORADRENALINE FROM SYMPATHETIC NEURONES IN RABBIT ISOLATED EAR ARTERY

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Cyclic AMP may possibly be involved in the stimulation-evoked release of transmitter from postganglionic sympathetic neurones (Göthert & Hentrich, 1984). The purpose of the present investigation was to study the possible role of intraneuronal cyclic AMP in the stimulation-evoked release of noradrenaline from vascular sympathetic neurones *in vitro*. This was accomplished mainly by the use of agents with reputed actions similar to those of cyclic AMP or by influencing the amount of endogenous cyclic AMP.

Strips of rabbit ear artery were used. The method has been described in detail (Abrahamsen & Nedergaard, 1990). Strips were preloaded with [^{3}H]-noradrenaline (10^{-7}M). After a wash-out period the strips were then subjected to electrical-field stimulation (S_1-S_7; 1 Hz; 150 pulses; 0.5 msec; 200 mA) 35 min apart. S_3 was used as an initial control value (~ 100%). Tetrodotoxin (10^{-6}M) reduced the stimulation-evoked [^{3}H]-overflow by up to 78%.

Cumulative addition of cyclic AMP (10^{-5}-$3x10^{-4}$M) enhanced the stimulation-evoked [^{3}H]-overflow from ear arteries preloaded with [^{3}H]-noradrenaline by up to 40 %. This enhancement was not altered by the phosphodiesterase inhibitor AH 21 132 ($3x10^{-5}$M). Cumulative addition of adenosine (10^{-5}-$3x10^{-4}$M) also enhanced the stimulation-evoked [^{3}H]-overflow by maximally 39%. 8Br cyclic AMP and dibutyryl cyclic AMP are cell-permeable, non-hydrolysable analogues of cyclic AMP. Cumulative addition of 8Br cyclic AMP (10^{-5}-$3x10^{-4}$M), but not dibutyryl cyclic AMP (10^{-5}-$3x10^{-4}$M) enhanced the stimulation-evoked [^{3}H]-overflow by up to 39%. The time course of the effect of single concentrations of cyclic AMP and 8Br cyclic AMP on stimulation (S_3-S_7)-evoked [^{3}H]-overflow was examined. Cyclic AMP ($3x10^{-4}$M) caused a biphasic response: initial enhancement (S_4) followed by inhibition (S_7). 8Br cyclic AMP ($3x10^{-4}$M) caused an enhancement which was most marked initially (S_4; 39%). Direct stimulation of adenylate cyclase by forskolin (10^{-7}-$3x10^{-5}$M) enhanced the stimulation-evoked [^{3}H]-overflow by maximally 63%. AH 21 132 ($3x10^{-6}$-$3x10^{-5}$M) and ICI 63 197 (10^{-4}M), both phosphodiesterase inhibitors, enhanced the stimulation-evoked [^{3}H]-overflow by maximally 76% and 31%, respectively. Forskolin (10^{-6}M) did not alter these enhancements.

The results are compatible with the view that depolarization-evoked release of noradrenaline from sympathetic neurones in rabbit ear artery is associated with an increase in intraneuronal cyclic AMP.

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290P EVIDENCE SUPPORTING A ROLE FOR NITRIC OXIDE IN THE VAGALLY EVOKED INHIBITION OF INTRAGASTRIC PRESSURE

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There is a growing body of evidence supporting a role for nitric oxide as a mediator of non-adrenergic, non-cholinergic smooth muscle relaxation in the gastrointestinal tract (Desai *et al* 1991). The vagus nerve is known to mediate gastric relaxation and it has been suggested that this may, at least in part, be mediated by nitric oxide. We have recently demonstrated the existence of a vagal inhibitory pathway to the rat stomach which is resistant to hexamethonium (Scott *et al* 1990). We have examined the role of nitric oxide in this pathway using *in vivo* and *in vitro* techniques.

In vivo studies were performed on fasted male Wistar rats (500 g) under urethane anaesthesia (1.25 g/kg i.p.). Intragastric pressure (IGP) was measured via a saline filled catheter introduced into the stomach via the pylorus. Arterial blood pressure was monitored via the left carotid artery and drugs were administered via the right jugular vein. The peripheral cut end of the left vagus nerve was stimulated at the cervical level (20V., 20Hz., 20s., 1ms. pulse duration), the right vagus was left intact. *In vitro* experiments employed an isolated stomach preparation with an intact vagal supply, maintained in a modified Krebs-Henseleit buffer. Isometric tension was recorded during vagal nerve stimulation (parameters as above) and bath application of drugs.

Vagal stimulation in both preparations elicited the expected biphasic response consisting of a rapid onset excitation followed by a longer lasting inhibition. Hexamethonium (30 mg/kg i.v.) *in vivo* abolished the excitatory response and led to a gradual increase in basal tone, revealing hexamethonium resistant inhibitions of IGP. Mecamylamine (0.1mM) *in vitro* produced a similar effect on isometric tension and in both cases the inhibitory response was of a lesser duration. *In vitro*, nitro-L-arginine methyl ester (L-NAME) at 0.1mM, significantly increased basal tone by 10.07 ± 2.88% (n=7, mean ± s.e.mean) and caused a progressive reduction in the size of the vagally induced inhibitions to 62.15 ± 7.07% of control (n=7). Subsequent application of L-arginine (5mM) partially but significantly reversed the actions of L-NAME *in vitro*, restoring evoked inhibitions to 83.40 ± 8.96% of control (n=7). Similar effects were seen *in vivo*, vagally induced inhibitions being reduced to 61.64 ± 8.27% of control by 10 mg/Kg L-NAME i.v. (n=7), although it was not possible to demonstrate a statistically significant reversal by administration of excess L-arginine (up to 200 mg/Kg i.v.).

These data support a role for nitric oxide in the vagally induced relaxation of the rat stomach. The release of nitric oxide is brought about by vagal fibres which do not operate via nicotinic ganglionic receptors.

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291P CHANGES IN NEURONAL CONTROL OF AIRWAY SMOOTH MUSCLE AFTER EXPOSURE TO OZONE

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Acute ozone exposure leads to a number of transient changes in the lung, including influx of inflammatory cells and airway hyperresponsiveness. Acetylcholine stimulates M3 muscarinic receptors on airway smooth muscle (ASM), producing contraction and bronchoconstriction. Release of acetylcholine from efferent parasympathetic nerves is under the control of inhibitory M2 muscarinic receptors on the vagus nerves. There clearly is some involvement of the parasympathetic nervous system in ozone-induced hyperresponsiveness since this hyperresponsiveness is abolished by atropine or vagal cooling in dogs and human subjects (Holtzman, 1985). These experiments were carried out to determine whether ASM M3 and/or neuronal M2 muscarinic receptors still function normally after acute ozone exposure. Specific pathogen free guinea pigs were exposed either to air or to 2.0 ppm ozone for 4 hours. 24 hours later the guinea pigs were anaesthetized with urethane (1.5 g·kg^{-1} i.p.), tracheostomized, paralyzed with continuous infusion of suxamethonium, and ventilated with a constant volume respirator. Autonomic innervation was abolished by transsecting both vagus nerves and by administering guanethidine (10 mg·kg^{-1} i.v.). Pulmonary inflation pressure (Ppi), blood pressure and heart rate were monitored. Intravenous methacholine (1-10 µg·kg^{-1} i.v.) and electrical stimulation of the distal ends of the vagus nerves (15 or 2 Hz, 5 or 22 sec, 10V, 0.2 msec) produced bradycardia, and bronchoconstriction as measured by increases in Ppi. M3 receptors on ASM were not altered following ozone exposure since methacholine dose-response curves were identical in both groups of animals (max Ppi = 286.7 ± 37.6 mm H_2O - air versus 315.0 ± 60.6 mm H_2O - ozone). The function of neuronal M2 receptors was tested by comparing vagally-induced bronchoconstriction before (B1) and after (B2) gallamine or pilocarpine. Vagally-induced bronchoconstriction is potentiated by the selective M2 antagonist gallamine and inhibited by the selective agonist pilocarpine (Fryer & Maclagan, 1984). Gallamine (0.1-10 mg·kg^{-1} i.v.) produced a dose-related increase of vagally-induced bronchoconstriction in control animals (B2/B1 = 7.8 ± 1.4) that was significantly attenuated in ozone-exposed guinea pigs (B2/B1 = 3.5 ± 0.3). Vagally-induced bronchoconstriction was inhibited in a dose-related fashion by pilocarpine (1-100 µg·kg^{-1} i.v.) in control animals (B2/B1 = 0.5 ± 0.04) but not in ozone-exposed guinea pigs (B2/B1 = 0.9 ± 0.1). These data suggest that ozone exposure inhibits the function of the M2 muscarinic autoreceptors on the pulmonary vagus nerves, and may explain the vagal hyperresponsiveness observed following ozone exposure.

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292P ANTIGEN-INDUCED PULMONARY M_2 MUSCARINIC RECEPTOR DYSFUNCTION IN GUINEA-PIGS IS REVERSED BY HEPARIN AND POLY-L-GLUTAMATE

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Release of acetylcholine from vagal fibers in the airways is controlled by inhibitory muscarinic M2 receptors on the nerves. These M2 receptors are not functioning in antigen challenged guinea-pigs thus potentiating vagally-induced bronchoconstriction (Fryer & Wills-Karp, 1991). Antigen challenge causes eosinophils to migrate into the airways. Therefore, we hypothesized that one of the strongly positively charged eosinophil proteins (major basic protein or eosinophil cationic protein) might interact with the M2 receptor, acting as an endogenous inhibitor. To test this hypothesis, we attempted to reverse the effects of antigen challenge by binding up the positively charged eosinophil proteins with poly-anionic substances. Guinea-pigs were injected with either saline or ovalbumin (10 mg·kg^{-1}, i.p.) on days 1, 3, and 5. On days 20-23 they were exposed to an aerosol of 5% ovalbumin for 5 min·day^{-1} (sensitization alone does not alter M2 receptor function). On the following day, animals were anaesthetised with urethane (1.5 g·kg^{-1}, i.p.), tracheostomised, ventilated with a constant-volume ventilator, and paralyzed with a continuous infusion of suxamethonium. Pulmonary inflation pressure (P_{pi}), blood pressure and heart rate were monitored. Both vagi were cut. Electrical stimulation of the distal portions of the vagi (2-15 Hz, 0.2 msec, 5-30 V, 45 pulses per train) produced bronchoconstriction measured as an increase in Ppi and bradycardia. In control animals, stimulation of the neuronal M2 receptors by the agonist pilocarpine (1-100 µg·kg^{-1},i.v.) decreased vagally-induced bronchoconstriction by 72±2%. Conversely blockade of these receptors by the M2 antagonist gallamine (0.1-10 mg·kg^{-1},i.v.) increased it 550±21%. As previously reported, the effects of both of these drugs were significantly reduced in sensitized, challenged animals, indicating loss of M2 receptor function. In a separate group of animals either heparin (2000 units·kg^{-1}) or poly-l-glutamate (10 mg·kg^{-1}) was given intravenously during the experiment. In sensitized, challenged animals, both treatments caused a significant (approximately 40%) decrease in the P_{pi} response to vagal stimulation, with a peak effect 20 min after injection. Neither treatment affected responses in control animals. After heparin or poly-l-glutamate treatment, the effects of both pilocarpine and gallamine on the P_{pi} response to vagal stimulation were restored, demonstrating recovery of M2 receptor function. Thus antigen-induced pulmonary M2 receptor dysfunction can be reversed by either a poly-anionic polysaccharide (heparin) or a poly-anionic peptide (poly-l-glutamate). This suggests that a poly-cationic substance such as eosinophil major basic protein or eosinophil cationic protein may be responsible for antigen-induced pulmonary M2 receptor dysfunction.

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293P ATTENUATION BY FORMOTEROL OF TRACHEAL PLASMA EXUDATION BY TOPICAL BRADYKININ IN RAT TRACHEA *IN VIVO*

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Inflammatory mediators and inhaled anti-asthma drugs can act on the airway mucosal microvasculature. We have studied the effects of the bronchodilator drug formoterol (FORMOT), applied to the tracheal mucosa (topical) or given i.p. (systemic), on transudation of plasma exudate into the lumen of the trachea in rats (anaesthetised with Hypnorm vet^R and midazolam) produced by mucosally-applied bradykinin (BK).

A tracheal segment (approx. 1 cm) was perfused in situ (0.1 ml/min) with normal saline containing captopril and thiorphan (10 μM). Perfusate, collected every 5 min, was assayed for plasma content using native fluorescence of plasma proteins (Miller-Larsson & Brattsand, 1991). In control experiments, two responses to bradykinin (BK1 and BK2, 20 μM, 5 min contact, 90 min apart) were not significantly different (paired t test, mean ± s.e.mean, μl plasma during 45 min; BK1, 11.4 ± 1.98; BK2, 9.6 ± 1.44, n=4). The response to BK2 was suppressed, compared to BK1, if FORMOT (10μM) was perfused through the trachea for 30 min before and also during the BK2 response (BK1, 11.9 ± 1.37; BK2, 5.6 ± 1.32, n=4, $0.01>P>0.001$). If FORMOT (10 μM) was perfused likewise but during the BK1 response (BK1, 3.5 ± 0.89; BK2, 5.6 ± 1.46, n=4) all BK1 and 3/4 BK2 responses were less than in control experiments. The response to BK2 was suppressed by systemic FORMOT (3 to 300 μg/kg i.p., given 60 min before the BK). The BK response was not abolished even after 300 μg/kg FORMOT; the reason for this is not yet clear. The BK1 response was significantly attenuated compared with the control experiments when FORMOT (100 μg/kg, n=4) was given before the BK1 response (BK1, 3.4 ± 0.33; $0.01>P>0.001$, Student's t test). The BK2 was also less but not significantly (7.7 ± 0.35, n=4). The effect of 10 μg/kg FORMOT on the BK2 response was prevented by 1 mg/kg propranolol i.v. 5 min before FORMOT (n=4).

In summary a) plasma exudation to topical BK in rat trachea can be suppressed by systemic or topical FORMOT b) the effects of systemic FORMOT involve β-adrenoceptors and c) responses to BK return towards normal 120 min after topical and 150 min after systemic FORMOT.

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294P SALMETEROL INHIBITS NEUTROPHIL ACCUMULATION IN RAT LUNG

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Salmeterol, a long-acting β_2-adrenoceptor agonist, has been shown previously to have anti-inflammatory properties in the guinea-pig, inhibiting both plasma protein extravasation (Whelan and Johnson, 1990) and the accumulation of granulocytes induced by platelet activating factor (Whelan and Johnson, 1991) or antigen (Sanjar *et al*, 1991). We have now investigated whether salmeterol also exhibits anti-inflammatory activity in rat lung, in a species in which β-adrenoceptor agonists are only weak bronchodilators. In the present study, we have compared the effects of salmeterol with those of the short acting β_2-adrenoceptor agonist, salbutamol.

Groups of male Wistar rats (~300g; ≥4) were exposed to aerosols of β_2-adrenoceptor agonists (nebulizer concentration, 0.01-30mM) or vehicle for 2min (Whelan and Johnson, 1990). After 30min, the animals were challenged with aerosolized lipopolysaccharide (LPS, 100μgml^{-1}) for 10min. Four hours later, the rats were killed (pentobarbitone, i.p.) and the lungs lavaged twice with 5ml phosphate-buffered saline, containing heparin, 10Uml^{-1}. Total and differential leucocyte counts were performed on aliquots of the bronchoalveolar lavage fluid (BALF) and the neutrophil content calculated.

In vehicle-treated rats, LPS increased the neutrophil content of BALF from 1.1 ± 0.7 to 250.9 ± 36.4 x 10^3 neutrophils. ml^{-1} (mean ± s.e.mean, n=4), 4h after challenge. However, pretreatment of the animals with aerosolized salmeterol caused a dose-related decrease in LPS-induced lung neutrophilia, with 31 ± 6 and 61 ± 5% inhibition being observed at 0.1 and 1.0mM (nebulizer concentration) respectively. In contrast, salbutamol had no marked effect on neutrophil accumulation, even at nebulizer concentrations of up to 30mM.

Furthermore, DL-propranolol (5mgkg^{-1} s.c.) administered 30 min before exposure to aerosolized salmeterol (1mM) reduced the inhibition from 62±10% to -1±13%. In contrast, following administration of D-propranolol (5mgkg^{-1} s.c.) aerosolized salmeterol (1mM) still caused 57±11% inhibition compared with 61±13% in control animals.

Thus, salmeterol, but not salbutamol, inhibits LPS-induced neutrophil infiltration into rat lung by an apparently β-adrenoceptor-mediated mechanism. These findings are a further confirmation of the anti-inflammatory properties of the long-acting β_2-adrenoceptor agonist, salmeterol.

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295P EFFECTS OF (+) AND RACEMIC SALBUTAMOL ON AIRWAY RESPONSES IN THE GUINEA-PIG

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In recent years, the incidence and severity of asthma, as well as associated death rates, have increased in several countries. It is appropriate therefore to ascertain whether anti-asthma drugs exhibit adverse effects that might contribute to these changes. An association between usage of beta-adrenoceptor agonist drugs and airway hyperreactivity in clinical asthma (Anonymous, 1990) has prompted study of (±)salbutamol, the most commonly used bronchodilator.

In the anaesthetised ventilated guinea-pig (Sanjar et al., 1990), reactivity of the airways to intravenous histamine (1.0-3.2 μg/kg) was enhanced significantly ($p < 0.01$, n=10,) following an intravenous infusion for one hour of (+)salbutamol (100 μg/kg), the non-bronchodilator enantiomer of racemic salbutamol. In studies with racemic salbutamol the bronchodilator action of (-) salbutamol precluded demonstration of airway hyperreactivity; hence, airway hyperreactivity was not detected following infusion of (±)salbutamol over 1 hour (100 μg/kg, n=10). However, increased responsivity to histamine was demonstrable four days after sustained subcutaneous infusion of (±)salbutamol (1 mg/kg/day, n=10), implying that the effect of (+)salbutamol on airway responsivity was less prone to tachyphylaxis than the spasmolytic effect of (-)salbutamol.

Subcutaneous infusion of (±)salbutamol (1 mg/kg) for more than two days increased the susceptibility of sensitised guinea-pigs to inhaled ovalbumin and caused almost 100 % mortality; an effect which was abrogated by inhalation of aerosolised (±)isoprenaline (0.1 % w/v) or subcutaneous injection of (±)salbutamol (1 mg/kg), immediately prior to inhalation of ovalbumin. Following subcutaneous infusion of (±)salbutamol (1 mg/kg, n=10) for 5 days, increased obstruction of the airways during inhalation or intravenous injection of ovalbumin was evident, which could account for death in such animals. Whether an increased incidence of neutrophils in the airway lumen observed 24 hours after inhalation of salbutamol (Boubekeur et al., 1989) contributed to the observed increase in airway reactivity has yet to be determined.

The capacity of (±)isoprenaline to induce airway hyperreactivity has been reported previously (Sanjar et al., 1990) and provides a plausible mechanism to account for the epidemic of asthma deaths twenty years ago (Speizer et al., 1968). In light of contemporary clinical evidence that bronchodilator therapy can be associated with enhanced airway reactivity, the pharmacology of (+)salbutamol and other (+)isomers of substituted catecholamines merits clinical investigation.

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296P NITRIC OXIDE AND ACETYLCHOLINE HYPERPOLARIZE SMOOTH MUSCLE CELLS IN THE RAT SMALL MESENTERIC ARTERY BY DIFFERENT MECHANISMS

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Acetylcholine and related cholinomimetics stimulate endothelium-dependent hyperpolarization and relaxation in arterial smooth muscle cells (Bolton et al, 1984; Taylor & Weston, 1988; McPherson & Angus, 1991). The differential sensitivity of the hyperpolarization and relaxation to various blocking agents has led to the suggestion that these events are mediated by separate endothelium-derived factors (Taylor & Weston, 1988). Recently, Tare & co-workers (1990) have demonstrated that nitric oxide, which appears to be or is closely related to EDRF, can stimulate smooth muscle hyperpolarization as well as relaxation, implying a role for nitric oxide in the endothelium-dependent hyperpolarization to acetylcholine. The present study investigated and compared the responses to both acetylcholine and nitric oxide in the rat mesenteric artery in a myograph.

Smooth muscle cells in isolated segments of rat small mesenteric artery had a resting potential around -57mV. Both acetylcholine and nitric oxide stimulated concentration-dependent hyperpolarization. The hyperpolarization to acetylcholine was endothelium-dependent, and increased the membrane potential to around -67mV. If the artery was first exposed to noradrenaline (1-3μM), the smooth muscle cells contracted, and were depolarized to -35mV. Acetylcholine again hyperpolarized the membrane to around -67mV with the highest concentration tested (3μM) and in addition, reversed the contraction by over 90%. Both the hyperpolarization and the relaxation were unaffected by the presence of glibenclamide (3μM). Nitric oxide (0.1-1μmole), applied either as a gas in solution or released from acidified sodium nitrite, produced a transient hyperpolarization of the resting membrane potential which varied between 3 and 9mV. Unlike acetylcholine, the hyperpolarization was abolished by prior smooth muscle depolarization in the presence of noradrenaline, although at this time nitric oxide stimulated marked smooth muscle relaxation. Glibenclamide (3μM) reversably blocked the hyperpolarization of the resting membrane potential which occurred in response to nitric oxide.

These data show that the smooth muscle hyperpolarizations to acetylcholine and nitric oxide are induced in different ways. The voltage-dependent block of hyperpolarization to nitric oxide suggests the involvement of inwardly-rectifying potassium channels, which because of their sensitivity to glibenclamide may be ATP-dependent.

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297P THE EFFECT OF BRL 38227 ON TENSION AND INTRACELLULAR CALCIUM LEVELS IN ISOLATED SMOOTH MUSCLE OF BOVINE TRACHEALIS

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Potassium channel openers (KCOs) inhibit a variety of agonist-induced contractions of smooth muscle and have recently been shown to reduce the intracellular calcium signal produced by low concentrations of KCl in vascular smooth muscle (Yanagisawa et al., 1990). In the present study we investigated the hypothesis that a reduction in intracellular calcium levels may underlie the relaxant effects of BRL 38227 (the active enantiomeric component in cromakalim) in acetylcholine (ACh)-stimulated bovine trachealis muscle.

Isolated strips of bovine trachealis were pre-contracted with ACh (3μM). Concentration-effect curves to BRL 38227 (0.03-10μM) were constructed and the maximally effective concentration of BRL 38227 was ascertained for use in the calcium measurements. The effect of glibenclamide (10μM) on the relaxant response to BRL 38227 was determined. Similar strips of trachealis which had been loaded overnight at room temperature with the calcium-sensitive dye Fura-2 (5μM with 0.02% cremophor) were used in the epifluorescence studies. The intracellular calcium levels were determined by measuring the ratio of the fluorescence signals produced by excitation at 340/380nm. Following determination of the basal ratio, tissues were exposed to ACh (3μM) for 10 min. After washout tissues were re-exposed to ACh (3μM) until a plateau response was achieved. BRL 38227 (10μM) or vehicle was added and the change in calcium signal observed. The effect of 45 min pre-incubation with glibenclamide (10μM) on the response to BRL 38227 was determined.

ACh (3μM) produced a marked contraction of bovine trachealis which was relaxed in a concentration-dependent manner by BRL 38227. Maximum relaxation of 57.7 ± 6.5% (n=6; mean ± s.e. mean) was obtained with 10μM BRL 38227. Glibenclamide significantly inhibited the relaxant response to BRL 38227. In the Fura-2 studies, ACh (3μM) produced an increase in the signal ratio (0.15 ± 0.01 ; mean± s.e.mean; n=18) which was maintained throughout the duration of ACh exposure (10-15 min). A second exposure to ACh produced a similar increase in the fluorescence ratio, the magnitude of which was assigned a value of 100%. Addition of BRL 38227 (10μM) reduced the calcium signal (maximum reduction 57.1± 5.7% ; n = 10; mean ± s.e.mean). Washout returned the signal to resting levels. Glibenclamide (10μM) significantly inhibited the response to subsequently added BRL 38227. Neither BRL 38227 nor glibenclamide changed the tissue autofluorescence. In contrast a small increase in autofluorescence of unloaded tissues was produced by ACh.

Clearly BRL 38227 reverses both the contraction and the rise in intracellular calcium produced by ACh in a glibenclamide-sensitive manner. These results provide support for a role of KCOs in opposing intracellular calcium signals produced by excitatory agonists.

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298P IDENTIFICATION OF CAPRIC ACID AS A POTENT VASODILATOR OF HUMAN AND CANINE BASILAR ARTERIES

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Certain saturated and unsaturated fatty acids (FA) produce diverse biological effects: uncouple phosphorylation, inhibit adenylate cyclase, inhibit Na-K ATPase, stimulate particulate guanylate cyclase and induce sleep. FA are also present in high tissue concentrations (as much as 12mM) in metabolic diseases and following cerebral ischemia or electroconvulsive shock. Moreover, arteries synthesize de novo and store free FA, and Holmquist and Ingvar (1957 have reported that butyrate (C4) or caprylate (C8) given intravenously could double cerebral blood flow in cats.

To ascertain whether FA (other than arachidonate) had a direct effect on arteries, the Na salt of C4, C6, C8, C10, C12, C14, C16 and C14:1, C18:1, C18:2 and C18:3 at concentrations of 4, 40, 400 and 4000 μM were applied to isolated segments of human basilar and umbilical arteries, and to isolated canine basilar and femoral arteries. In addition, the effect of C8 and C10 (caprate) on canine femoral blood flow was determined.

In most experiments the isolated arteries were precontracted with prostaglandin $F_2\alpha$ (EC_{85}) and then a FA was added. The cerebral arteries were more sensitive to all FA than the peripheral vessels and C10 was the most potent. The EC_{50} for C10 with the canine basilar artery was 49μM and with the human basilar artery 63μM, indicating no species difference. The EC_{50} for C10 in the femoral and umbilical arteries was 440 μM and 780 μM, respectively. Contractions elicited with equieffective concentrations of U46619, serotonin and prostaglandin $F_2\alpha$ were similarly inhibited by C10. The dilator response did not manifest desensitization or tachyphylaxis. Among the unsaturated FA, monounsaturated (C14:1, C18:1) were more potent than the polyunsaturated (C18:2, C18:3) on basilar arteries, although far less potent than caprate. The effect of C10 on Ca-induced contractions of arteries first placed in Ca-free buffer and depolarized with KCl indicate that C10 does not inhibit Ca-influx to relax the vessels; nor does removal of the endothelium affect the vasorelaxation. Caprate at 1, 3, 5 and 10 μmol/kg intra-arterially increased femoral blood flow in a dose-dependent manner, reversed NE constriction, and was more effective than C8 as a vasodilator. The dose computed to raise the flow 50% was 1.27 μmol/kg, suggesting that the arterioles may be more sensitive to C10 than the parent femoral artery. At the doses studied there was no systemic effect from the intra-arterial injection and no tachyphylaxis. The results indicate that FA could affect vasomotion in health or disease and that C10 could be a useful agent to increase blood flow to specific vascular beds experimentally and therapeutically.

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299P FLOSEQUINAN RELAXES STRETCH-INDUCED TONE AND CONTRACTIONS INDUCED BY KCL, PHORBOL DIBUTYRATE AND NORADRENALINE IN ISOLATED HUMAN ARTERY AND VEIN STRIPS

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Flosequinan (Fq) is a vasodilator with a novel chemical structure that was recently shown to have two different effects on intracellular Ca ([Ca++]i) in animal vessels: a generalized decrease of [Ca++]i after KCl and stretch/temperature-induced contraction and the reversal of the sensitization of the myofilaments to [Ca++]i in phenylephrine-mediated vasoconstriction (Resnick et al., 1991). We evaluated the effect of Fq on the contractions induced in human vessels strips by: 1) KCl 10 mM, which increases [Ca++]i but decreases the calcium sensitivity of myofilaments; 2) by noradrenaline (NA), which increases [Ca++]i and sensitizes myofilaments to Ca++; 3) by phorbol dibutyrate (PDB) constrictor activity which is calcium-independent; 4) by stretch-induced tone (SIT), which increases the permeability to extracellular calcium (EGTA-sensitive). The vessels were detached from the adventitial layer and cut into helical strips about 1 cm long. The strips were placed in a thermostated perfusion bath at 37°C and connected to a force-displacement transducer. The perfusion medium consisted of 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_3, 2.5 mM $CaCl_2$, 11 mM D-Glucose, pH 7.4, gassed with 95%O_2/5%CO_2. Stable contractions were induced in coronary strips (obtained from patients undergoing cardiac transplantation for idiopathic dilated cardiomyopathy) and increasing doses of Fq (1-100 μM) added. Fq 10 μM (a clinically-relevant concentration, Packer et al., 1988) relaxed contractions to 10 mM KCl, 1 μM PDB, 100 μM NA and SIT by 62 ± 6%, 43 ± 11%, 31 ± 4% and 76 ± 12% respectively (n = 4). The dose response curve to NA in mammary artery strips (EC_{50} 0.1 ± 0.01 μM) was shifted to the right in a non-competitive manner by pretreatment with 10 μM Fq (EC_{50} 0.7 ± 0.06 μM; $P < 0.01$). Finally, saphenous vein strips (n = 5) developed sustained tonic contractions, in some instances with phasic waves, after a loading stretch (0.5 - 1 g). The tension was inhibited by EGTA and papaverine (1 μM) consistent with stretch-induced tone. Fq (10 μM) relaxed the stretch-induced tonic contractions (from 25 to 76% of the maximal contraction). In those vessels demonstrating both phasic and tonic contractions Fq inhibited mainly the tonic ones. In conclusion, Fq at clinically-relevant concentrations (10 μM) is an effective relaxant of human artery and vein strips. The antagonizing effect of Fq on absolute [Ca++]i and on the Ca++-sensitization of the myofilaments are the most likely explanation of its vasodilator effect in human vessels also.

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300P GR117289: A NOVEL, POTENT AND SPECIFIC NON-PEPTIDE ANGIOTENSIN RECEPTOR ANTAGONIST

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GR117289 (1-[[3-Bromo-2-[2-(1H-tetrazol-5-yl)phenyl]-5-benzofuranyl]methyl]-2-butyl-4-chloro-1H-imidazole-5-carboxylic acid) is one of a series of recently synthesised non-peptidic compounds which antagonise the actions of angiotensin II (Ang II) both *in vitro* and *in vivo* (Middlemiss et al., 1991). The present study addressed the effects of GR117289 *in vitro* using the rabbit aorta, which contains angiotensin receptors (AT_1 or Type B) sensitive to blockade by the non-peptide, competitive antagonist, DuP753 (Wong et al., 1990a).

Helical strips (1.5-2cm) of thoracic aorta from male, New Zealand White rabbits were suspended under a resting tension of 0.5g in Krebs solution at 37°C, containing indomethacin (30μM) and ascorbic acid (100μM), and gassed with 95% O_2 / 5% CO_2. Cumulative, concentration-response curves to Ang II (0.1-100nM) were constructed until reproducible. Subsequently, GR117289 (0.3-3nM ; 45min incubation) caused concentration-related, insurmountable suppressions of the Ang II concentration-response curves. Longer incubation times produced a more marked suppression of the Ang II curve suggesting that GR117289 was slow to reach equilibrium. After 3h incubation, an apparent pK_B for GR117289 of 9.8 ± 0.1 (n=7) was derived from a double-reciprocal regression plot (Kenakin, 1984). In separate experiments, Ang II concentration response curves were again constructed before and after exposure to GR117289 (0.3-3nM ; 45min incubation). The tissues were then superfused with Krebs solution devoid of GR117289 for 3h, and then rechallenged with Ang II. Washing did not reduce the extent to which the responses to Ang II were suppressed. In preparations treated with GR117289 (1nM ; 2h 45min incubation), in which responses to Ang II were markedly suppressed, co-incubation with DuP753 (10, 100 and 1000nM ; for the last 45min) caused a concentration-dependent, upward and rightward displacement of the concentration-reponse curve to subsequently administered Ang II. These data suggest that, although GR117289 cannot be easily washed out from the tissue, it is not irreversibly bound to its receptor. GR117289 (1μM) caused a less than 2-fold displacement of the concentration-response curves to either phenylephrine (10nM-30μM) or 5-HT (10nM-10μM). In conclusion, GR117289 is a potent, specific, slowly reversible, non-peptide antagonist at AT_1 receptors in the rabbit aorta. The profile of action of GR117289 in this tissue resembles that of EXP3174, the recently described acid metabolite of DuP753 (Wong et al., 1990b).

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301P THE ROLE OF PLD CATALYSED PtdCho HYDROLYSIS IN SUSTAINED DIACYLGLYCEROL FORMATION IN VASOPRESSIN-STIMULATED A10 VASCULAR SMOOTH MUSCLE CELLS

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In cultured vascular smooth muscle cells (VSMCC) a number of vasoconstrictors such as angiotensin II and endothelin-1, stimulate the biphasic formation of *sn*-1,2 diacylglycerol (DAG) (Sunako *et al.*, 1990). Whilst the initial phase of DAG is formed from the breakdown of PtdIns(4,5)P_2 , the second more prolonged phase is believed to be derived from the hydrolysis of another phospholipid, phosphatidylcholine (Billah and Anthes 1990). In a previous communication we showed that vasopressin (VP) stimulated PtdCho hydrolysis in A10 VSMCC through intermediate activation of protein kinase C (PKC) (Plevin *et al.*,1991). We have now sought to determine if PtdCho was the major source of vasopressin-stimulated sustained DAG formation in these cells by comparing the kinetics of PtdCho hydrolysis and DAG formation.

Phosphatidylcholine hydrolysis was measured in two ways: 1) analysis of water soluble choline metabolites in cells prelabelled with [^{3}H]choline (5μCi/ml) and 2) the accumulation of phosphatidylbutanol ([^{3}H]PtdBuOH) in [^{3}H]palmitate prelabelled cells (5μCi/ml), incubated in the presence of 30mM butanol. Agonist-stimulated *sn*-1,2 DAG mass formation was measured as outlined by Paterson *et al.*,(1991).

In A10 VSMCC, vasopressin-stimulated [^{3}H]PtdBuOH accumulation was found to be transient, reaching a peak between 2-5min. In cells pretreated with vasopressin (5min) then incubated with butanol to trap any phosphatidyl moieties generated, no accumulation of PtdBuOH was observed (% increase in [^{3}H]BuOH:vehicle pretreatment = 684.6±8.8, vasopressin pretreatment = 8.4±1.1, n=3), suggesting that agonist-stimulated PLD activity was desensitized. Vasopressin also stimulated a transient increase in intracellular choline formation which reached a peak at 30 sec (d.p.m; control= 385±21, vasopressin =1221±57, n=5) and returned to basal values within 5 min. No increase in the PLC derived metabolite, choline phosphate was observed, suggesting that PLD is the major catalytic route of PtdCho hydrolysis in these cells. Vasopressin-stimulated a biphasic increase in DAG which was sustained for up to 60min (pmol/sample; 5min control=34.3±8, VP =134±14.9; 60min, control=39.0±5.0 VP=79.3±7.5, n=4). PMA also stimulated DAG formation, but to a lesser extent than vasopressin and with single phase kinetics. However, whilst the PMA response was inhibited by 70-80% by either butanol pretreatment which inhibits the formation of phosphatidic acid from PLD hydrolysis, or PKC inhibition (Ro-31-8220 100μM), vasopressin-stimulated DAG formation was reduced only approximately 40% and 30% by these interventions. (DAG pmol/sample:control= 60.6±10.0, VP=145.0±15.3, VP+but(0.3%)=99.5±5.5 ; control=43.2±8.0, VP=178±15.1, VP+Ro =137.0±14.0, n=3, 10 min stimulation).

The rapid and transient nature of vasopressin-stimulated PtdCho hydrolysis is inconsistent with its proposed role as the source of sustained DAG. Furthermore, inhibition of PLD activated PtdCho hydrolysis resulted in only partial reduction in vasopressin-stimulated DAG formation. It seems likely therefore, that an additional pathway is involved in sustained DAG generation in smooth muscle cells.

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302P ATP AND ITS METABOLITE ADENOSINE ACT SYNERGISTICALLY TO MOBILIZE INTRACELLULAR CALCIUM VIA THE FORMATION OF INS(1,4,5)P_3 IN A SMOOTH MUSCLE CELL LINE

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Interactions between ATP- and adenosine-receptors on the formation of Ins(1,4,5)P_3, as measured by a competitive binding assay, and mobilization of intracellular calcium, as determined with the Fura-2 technique, were investigated in the smooth muscle cell line DDT_1 MF-2. Activation of adenosine A_1 receptors with cyclopentyladenosine (CPA) or $P_{2(Y)}$ receptors with ATP increased both Ins(1,4,5)P_3 formation and intracellular calcium concentrations. The A_1 receptor induced Ins(1,4,5)P_3 formation was antagonized by the A_1 antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and by pretreatment of the cells with pertussis toxin (PTX). ATP effects were partially affected by PTX treatment. ATP and CPA had supraadditive effects on Ins(1,4,5)P_3 accumulation and CPA increased ATP induced Ins(1,4,5)P_3 accumulation in a concentration-dependent manner with an EC_{50} of 3 nM, a concentration that per se had little or no effect on Ins(1,4,5)P_3 accumulation.

ATP (EC_{50} 4 μM) and CPA (EC_{50} 3 nM) both increased intracellular calcium levels. The effect of ATP was partially sensitive to PTX treatment, whereas the effect of CPA was blocked both by PTX and by DPCPX. Concentrations of ATP and CPA that by themselves were insufficient to raise intracellular calcium were able to do so when combined. CPA and ATP did not act synergistically after PTX treatment of the cells.

Since ATP was metabolized, by ecto-enzymes, to ADP, AMP and adenosine, we further examined if adenosine formed from ATP could enhance the ATP effects on Ins(1,4,5)P_3 accumulation. Indeed, the addition of the A_1 receptor antagonist DPCPX or the removal of endogenous adenosine by adenosine deaminase significantly attenuated the ATP response and the two treatments did not have additive effects.

The present study thus demonstrates that in a clonal cell line two types of purinergic receptors increase phospholipase C activity, but via different mechanisms: P_2 receptors appeared to act via partially PTX-insensitive, and A_1 receptors via PTX-sensitive G-proteins. ATP and CPA are not only able per se to induce formation of Ins(1,4,5)P_3 and mobilize intracellular calcium, but they also act synergistically. Finally, it is demonstrated that endogenous adenosine, presumably formed from the rapid breakdown of ATP, can significantly enhance the ATP effects.

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303P THE NEUROPROTECTIVE EFFECT OF THE KAPPA AGONIST CI-977 IN A RAT MODEL OF FOCAL CEREBRAL ISCHAEMIA

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Agonists at the kappa opioid receptor are putatively beneficial in some animal models of cerebral ischaemia. The mechanistic basis for the neuroprotective effects of kappa agonists is unknown. Although attenuation of excitotoxic mechanisms or cerebral circulatory effects may contribute to the effects of kappa opioids, the ability of the drugs to reduce cerebral oedema has attracted the greatest speculation.

CI-977 is a highly potent and selective kappa opioid receptor agonist (Hunter et al., 1990). We have investigated the ability of CI-977 to reduce ischaemic brain damage and brain swelling in a model of focal cerebral ischaemia.

The left middle cerebral artery (MCA) was permanently occluded via a subtemporal approach during a brief period of halothane anaesthesia (Tamura et al., 1981) in 28 male Sprague-Dawley rats (340-390g). The animals were sacrificed 24h after MCA occlusion. The brains were removed, multiple sections taken throughout the cerebrum and stained with haematoxylin-eosin. Areas of ischaemic damage and of brain swelling were assessed using an image analyser at 8 preselected coronal planes. Volumes of ischaemic damage and brain swelling were computed from the area and the known stereotactic co-ordinates of the planes.

CI-977 produced a dose-dependent reduction in the volume of ischaemic damage which was most marked at 0.3mg/kg (controls 188 ± 11mm^3; CI-977-treated 117 ± 21mm^3, $P<0.02$ t-test with Bonferroni correction). In the same animals, CI-977 reduced the amount of brain swelling to a similar extent (controls 140 ± 6mm^3; CI-977, 0.3mg/kg, 98 ± 10mm^3, $P<0.002$). There was an excellent correlation between the volumes of brain swelling and ischaemic damage which was similar with saline-treated and CI-977-treated animals (overall correlation coefficient = 0.896).

These results indicate that CI-977 is effective in reducing ischaemic brain damage in a model of focal cerebral ischaemia and that the reduction in brain swelling is a consequence of reduction in ischaemic damage.

K.B. Mackay is an MRC/CASE student.

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304P THE NEUROPROTECTIVE ACTIONS OF LIPOCORTIN-1 IN CEREBRAL ISCHAEMIA IN THE RAT

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Lipocortins (annexins) are a family of endogenous calcium and phospholipid binding proteins proposed as mediators of the ant-iinflammatory actions of glucocorticoids, possibly acting by inhibition of arachidonic acid production (Flower, 1988). Lipocortin-1 (LC-1) has been shown to be present throughout the rat brain (Strijbos et al, 1991) and has been proposed as an endogenous inhibitor of cerebral ischaemia (Relton et al, 1991). The objective of this study was to determine the time course of action of LC-1 following cerebral ischaemia in the rat, and to investigate possible mechanisms for its neuroprotective actions.
Male Sprague-Dawley rats (200-300g) were stereotaxically implanted with guide cannulae into the third ventricle of the brain 3-4d prior to experimentation. Cerebral ischaemia was induced by permanent unilateral occlusion of the middle cerebral artery (MCAo) under halothane anaesthesia. Infarct size was determined from tetrazolium staining 24h after MCAo and results (lesion volume, mm^3), quantified using Seescan image analysis.

Intracerebroventricular (icv) injection of an active fragment of LC-1 (N-terminal 1-188aa)(1.2μg in 3μl), 30min before and 10min after the induction of ischaemia resulted in a 71.4 ± 8.5% reduction ($n=10$, $p<0.01$) in infarct volume assessed 24h later. When a single dose of LC-1 fragment was administered either 10, 30 or 60min after ischaemia reductions in infarct volume of 52.9 ± 7.6% ($n=10$, $p<0.05$) 38.5 ± 10.1% ($n=10$, $p<0.05$) and 27.0 ± 18.3% ($n=10$) respectively were observed. In separate experiments intact animals were treated with either the synthetic glucocorticoid, dexamethasone (50μg/rat, sc, 36 and 12h prior to MCAo) or the cyclooxygenase inhibitor ibuprofen (10mg/kg, sc, 30min before and 10min after MCAo). Ibuprofen caused a small but not significant reduction in infarct volume (21.4 ± 16.3%, $n=8$) whereas dexamethasone slightly potentiated neuronal damage (48.5 ± 8.3% increase in infarct volume, $n=6$).

These findings indicate that LC-1 is a potent inhibitor of neuronal damage following cerebral ischaemia in the rat, which can provide neuroprotection when a single dose is administered up to 1h after the induction of ischaemia. The results reported here also suggest that the mechanism of action of LC-1 is unrelated to its involvement in the anti-inflammatory actions of the glucocorticoids, or by modification of prostaglandin synthesis. Other possible mechanisms of action of LC-1 include calcium sequestration, inhibition of free radical production or inhibition of the excitotoxic actions of glutamate at the NMDA receptor.

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305P LIPOCORTIN-1 INHIBITS NMDA RECEPTOR-INDUCED NEURONAL DAMAGE

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The involvement of the NMDA receptor in neuronal damage produced by cerebral ischaemia has been well documented (Choi and Rothman, 1990). Lipocortin-1 (annexin-1) an endogenous phospholipid and calcium binding protein has been shown to significantly attenuate the damage produced by focal cerebral ischaemia in the rat (Relton et al., 1991). In the present study we have therefore investigated its effect on NMDA induced neuronal damage.

Unilateral intrastriatal infusion (10nmoles in 1μl) of a potent and selective NMDA agonist, cis 2,4-methanoglutamate (MGlu) (Lanthorne et al., 1990), induced an extensive lesion of the striatum in the rat. Lesion size was determined using a stain for mitochondrial viability (tetrazolium blue) after 24h, and results quantified using a Seescan image analysis system. Intraperitoneal injection of MK801 (4mg/kg), 30min prior to infusion, reduced the extent of the lesion by 81.8 $\pm$ 6.9% (n=6, $p<0.001$). Co-infusion of the active fragment of lipocortin-1 (N-terminal 1-188aa) (1.2μg in 3μl) prior to 10nmol MGlu reduced the extent of damage by 44.2 $\pm$ 8.0% (n=9, $p<0.01$) when compared to control values. Furthermore coinfusion of 3μl of the neutralizing antibody raised to the lipocortin fragment with 5nmol MGlu increased the size of the lesion by 157.9 $\pm$ 22.0%. All infusions were made at a rate of 1μl min^{-1}. This antibody is known to bind both the fragment and endogenous form of lipocortin-1 (Carey, unpublished observations).

These findings indicate that the damage produced by intrastriatal infusion of MGlu is mediated by the NMDA receptor. The observation that the active fragment of lipocortin-1 attenuates, and the neutralizing antibody increases, this NMDA-mediated neuronal damage may explain its neuroprotective action following cerebral ischaemia.

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306P THE ROLE OF $GABA_B$ RECEPTORS IN TERMINATING EPILEPTIFORM ACTIVITY IN THE RAT ENTORHINAL CORTEX *IN VITRO*

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Epileptiform activity evoked by $GABA_A$ antagonists, in the rat entorhinal cortex (EC) *in vitro* arises initially in the deep layers (IV-IV) and then propagates to layer II (Jones and Lambert, 1990). It can spread to the dentate gyrus via the perforant path. but the dentate acts as a filter, restricting spread to the hippocampus proper. However, the epileptiform activity is also dampened down considerably in layer II, as discharges driven in this layer from the deep layers are curtailed by a strong hyperpolarizing afterpotential. The present experiments investigated the role of synaptic inhibition mediated by $GABA_B$-receptors in the manifestation of this hyperpolarizing potential.

Slices of EC from the rat brain were maintained *in vitro* at the interface between artificial CSF (at 34 °C) and moistened carbogen gas. Intracellular recordings were made from layer II neurones. Synaptic responses were evoked in these neurones by stimulating either the parasubiculum or in the deep layers of the EC. Epileptiform activity was provoked by perfusing with bicuculline methochloride (BMC, 2-5 uM). $GABA_B$-receptors were blocked with 2-hydroxysaclofen (2-OHS, 100-500 uM). Synaptic responses of layer II neurones in the absence of any convulsants are complex and exhibit components mediated by both N-methyl-D-aspartate (NMDA) and non-NMDA excitatory amino acid receptors as well as fast and slow IPSPs (Jones, 1990). Perfusion with 2-OHS greatly reduced the amplitude (81+/-7%) and duration (74+/-4%) of the slow IPSP (n=6) showing it to be largely $GABA_B$-receptor mediated. The fast IPSP was little affected and has previously been shown to be $GABA_A$-receptor mediated (Jones, 1990). Perfusion with BMC blocked the fast IPSPs (100%, n=56) and ultimately resulted in the generation of epileptiform discharges. BMC did not block the slow IPSP and in many cells (n=21) its amplitude and duration was greatly enhanced (by 36+/-9% and 28+/-14% respectively). When the epileptiform discharges reached a stable configuration they usually consisted of an initial paroxysmal depolarization (PD) followed by a variable number of afterdischarges. The PD was followed by a pronounced slow hyperpolarization and the afterdischarges were superimposed on this. Perfusion with 2-OHS (n=5) greatly reduced this slow hyperpolarization, enhanced the PD and increased the number of afterdischarges.

Thus, the slow IPSPs of layer II EC neurones are $GABA_B$-receptor mediated. During epileptogenesis provoked by blocking $GABA_A$-inhibition the $GABA_B$-inhibition is enhanced and plays a part in curtailing the epileptiform discharge and restricting its spread to the hippocampus.

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307P THE IMR-32 HUMAN NEUROBLASTOMA CELL POSSESSES A FUNCTIONAL $GABA_A$ RECEPTOR

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The $GABA_A$ receptor is a multimeric protein containing a chloride channel which opens on receptor activation. In addition to binding sites for agonists, the receptor also contains binding sites for allosteric modulators such as benzodiazepines. The chloride channel can be identified in radioligand binding studies using [^{35}S]-TBPS (t-butylbicyclophosphorothionate) or by measuring chloride flux. We have investigated whether $GABA_A$ receptors are present on human neuroblastoma (IMR-32) cells using both ligand binding and chloride flux measurements.

IMR-32 cells were maintained in culture with MEM supplemented by 10% foetal calf serum, 2 mM L-glutamine and 50 $\mu g ml^{-1}$ gentamicin. [^{35}S]-TBPS binding was carried out on freshly prepared washed membranes and [^{3}H]-flunitrazepam binding to frozen membranes (Cross et al 1989). Chloride efflux was measured by studying the reduction of quenching of the fluorescent dye 6-methoxy-N-3-sulphonopropyl-quinolinium (SPQ) by intracellular chloride. Dye was loaded into IMR-32 cells for 60 min at 37°C in buffer containing 10 mM SPQ and 150 mM chloride. Cells were transferred to low chloride buffer containing furosemide (1mM) and DIDS (10 μM). Chloride efflux increased fluorescence which was expressed as a percentage above basal fluorescence.

GABA (ED_{50} 10 μM) increased fluorescence maximally by 8.96+1.00%; this was blocked completely by picrotoxin (1μM). Pentobarbital (50-500μM) increased chloride flux alone and potentiated the effect of GABA. Flurazepam was without effect. [^{35}S]-TBPS binding sites were present in IMR-32 cell membranes (Kd=28 $\pm$ 5 nM, Bmax = 224 $\pm$ 47 fmol/mg protein) and were consistent with binding to a $GABA_A$ receptor (Table 1). No specific [^{3}H]-flunitrazepam binding was observed.

Table 1 Inhibition of [^{35}S]-TBPS binding in rat brain and IMR-32 cells

	$IC_{50}\mu M$			
	GABA	Muscimol	Picrotoxin	Pentobarbital
Rat cortex	8.3 $\pm$ 2.5	4.8 $\pm$ 1.1	0.45 $\pm$ 0.11	50 $\pm$ 6.1
IMR-32 cells	30 $\pm$ 6.2[a]	1.3 $\pm$ 0.6[a]	0.60 $\pm$ 0.11	255 $\pm$ 29

Values are mean $\pm$ s.e.mean, n = 3-4; a - maximal 45% inhibition.

We conclude that undifferentiated IMR-32 cells express a $GABA_A$ receptor-chloride channel, which is modulated by pentobarbital but not flurazepam. Thus the $GABA_A$ receptor in this cell line differs from that in rat cortex.

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308P BRAIN INDOLE AMINE METABOLISM DURING PORTAL-SYSTEMIC ENCEPHALOPATHY

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Portal-systemic encephalopathy (PSE) is characterised by liver dysfunction and portal-systemic diversion: it is important to differentiate between these two sub-components in order to rationalise effective clinical therapy. This study presents an experimental surgical model in the portacaval transposed rat (PCT) which can now make this possible. The PCT rat is a useful model for comparative evaluations since portal-systemic diversion is achieved with the retention of normal total hepatic blood flow and liver function unlike experimental models of PSE such as portacaval shunted (PCS) rats in which both sub-components are expressed. Studies in PCS rats which have suggested that alterations in brain indole amine metabolism may be responsible for the development of PSE (Bengtsson et al 1988), have not considered whether these changes may have occurred as a consequence of portal-systemic diversion or liver failure. This study proposed to answer this question since changes that are measured in PCS rats and not PCT rats are indicative of liver failure and not portal-systemic diversion. 80 Sprague-Dawley rats were subjected to PCS, PCT and sham-operation and investigated for 1, 3, 9 and 15 weeks. Intra-peritoneal Injection of the monoamine oxidase inhibitor NSD 10/15 (m-hydroxy benzylhydrazine) (100mg/kg) allowed measurements of the turnover of 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) to be made in the midbrain and cerebral regions of the 3 groups of rats.

Over the 15 week period studied, a gradual modification in 5-HT metabolism was observed. Only sustained changes in PCS but not in PCT rats at 15 weeks were measured in brainstem 5-HIAA and 5-HTP.

Cerebral and brainstem concentrations of brain indole amines in PCS, PCT and control rats

	PCS	PCT	Control
Brainstem 5-HIAA	0.68 $\pm$ 0.01***	0.37 $\pm$ 0.04	0.25 $\pm$ 0.01
Brainstem 5-HTP	0.29 $\pm$ 0.05*	0.22 $\pm$ 0.02	0.18 $\pm$ 0.02
Brainstem 5-HT	0.51 $\pm$ 0.02	0.43 $\pm$ 0.03	0.42 $\pm$ 0.03
Cerebral 5-HIAA	0.31 $\pm$ 0.03**	0.23 $\pm$ 0.01	0.18 $\pm$ 0.01
Cerebral 5-HTP	0.23 $\pm$ 0.02***	0.15 $\pm$ 0.01	0.17 $\pm$ 0.01
Cerebral 5-HT	0.36 $\pm$ 0.02	0.38 $\pm$ 0.02	0.39 $\pm$ 0.01

Results shown are μg / g brain, 15 weeks following the operations (mean $\pm$ sem, n = 6), *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, Student's unpaired t-test, shunted vs transposed rats. Significant elevations in cerebral 5-HTP and 5-HIAA, and in brainstem 5-HIAA suggested a reduced turnover of 5-HT in these brain regions of PCS rats compared to PCT rats: the significance of these in the pathogenesis of PSE remains to be clarified. It is proposed that the PCT rat is a useful model for differentiating changes observed during PSE which may be due to portal-systemic diversion alone.

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309P NEURONAL BUNGAROTOXIN IS A POTENT ANTAGONIST AT THE NICOTINIC RECEPTOR ON THE SOMATIC MUSCLE CELLS OF THE PARASITIC NEMATODE *ASCARIS SUUM*

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Neurotoxins distinguish between different nicotinic acetylcholine receptor subunit compositions expressed in *Xenopus* oocytes (Leutje *et al*, 1991). Neuronal bungarotoxin (Bungarotoxin 3.1) only blocks $\alpha_3\beta_2$ and possibly $\alpha_4\beta_2$ subunit combinations whereas α conotoxin only blocks subunit combinations containing the α_1 muscle type subunit. Previous work has demonstrated that the nicotinic receptor on the somatic muscle cells of *Ascaris suum* is pharmacologically more similar to a neuronal type nAChR than a muscle type (Colquhoun *et al*, 1990, 1991). This was investigated further using neuronal bungarotoxin and α conotoxin. Conventional two-electrode electrophysiological recording techniques were employed.

Neuronal bungarotoxin was a potent antagonist of ACh induced depolarisation and conductance change, (figure 1). 10nM nBTX blocked the response to a submaximal dose of ACh to 85±10% of control (n=3,±s.e.mean), 100nM nBTX blocked to 18±7% of control (n=4,±s.e.mean). α Conotoxin had no antagonistic action up to 10μM (n=3). These data confirm that the *Ascaris* nicotinic acetylcholine receptor on the muscle cells is neuronal like.

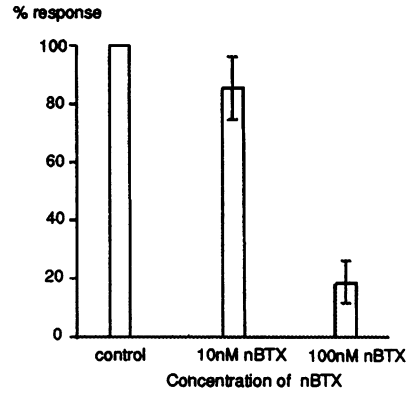

Figure 1. Control is conductance increase to ACh in absence of nBTX. Cells were incubated with toxin for 30 minutes.

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310P EVIDENCE THAT ECTO-5'-NUCLEOTIDASE ACTIVITY IN RAT HIPPOCAMPAL BUT NOT IN CEREBRAL CORTICAL SLICES IS ASSOCIATED WITH THE CHOLINERGIC NERVE TERMINALS

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Ecto-5'-nucleotidase mediates formation of adenosine from extracellular adenine nucleotides at the neuromuscular junction (Ribeiro & Sebastião, 1987). To investigate whether ecto-5'-nucleotidase activity is associated with cholinergic nerve terminals, we compared the activity of the enzyme in hippocampal and cortical immunopurified cholinergic nerve terminals with that obtained in slices from the same brain areas.

Rat brain cortical and hippocampal slices were 400 μm thick and were prepared as described (Sebastião et al., 1990). Immunopurified cholinergic nerve terminals were prepared according to Richardson et al. (1984). After immunopurification, the enrichement of cholinergic nerve terminals over the P2 fraction was 22-fold in the hippocampus and 16-fold in the cerebral cortex, as assessed by the choline acetyl transferase activity. AMP (10 μM) was added to the preparations (30°C) at zero time. Aliquots (60-70 μl) were removed from the bath at various times up to 30 min and analysed by reverse-phase HPLC (Cunha & Sebastião, 1991).

Catabolism of exogenously added AMP (10 μM) originated adenosine and inosine in hippocampal cholinergic nerve terminals, hippocampal slices and cerebral cortical slices. This AMP catabolism was inhibited (87±3%, range: 77-100%, n=8) by the ecto-5'-nucleotidase inhibitor α,ß-methylene ADP (100 μM), but unaffected by the substrate of phosphatases, p-nitrophenylphosphate (1 mM, n=5). The comparison of the relative rate of catabolism of AMP in hippocampal cholinergic nerve terminals (130±30 nmol/5 min/mg protein, n=6) with that found in hippocampal slices (2.5±0.5 nmol/5 min/mg protein, n=9) revealed a near 50-fold enrichement in ecto-5'-nucleotidase activity in cholinergic nerve terminals. The rate of AMP catabolism in cortical slices (0.8±0.2 nmol/5 min/mg protein) was 3 times lower than in hippocampal slices. Exogenous AMP (10 μM) was virtually not metabolized in the cortical cholinergic nerve terminals (n=4), though exogenous ATP (10 μM) was metabolized (77±8 nmol/5 min/mg protein, n=2) into ADP and AMP.

The results suggest that in the hippocampus there is an ecto-5'-nucleotidase activity preferentially associated with the cholinergic nerve terminals, whereas in the cerebral cortex ecto-5'-nucleotidase activity prefers localizations different from the cholinergic nerve terminals.

The gift of the immunoadsorbent and antiserum by Dr. P.J. Richardson is acknowledged. R.A.Cunha is supported by JNICT.

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311P COUPLING OF ADENOSINE A_1 AND A_2 RECEPTORS TO G-PROTEINS AS STUDIES WITH AUTORADIOGRAPHY

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There are two major classes of adenosine receptors A_1 and A_2 that are coupled via G_i and G_s-families of G-proteins, respectively, to adenylate cyclase. In the present study we have examined how A_1-receptor agonist [^{3}H]-cyclohexyladenosine (CHA) and antagonist [^{3}H]-1,3-dipropyl-8-cyclophenyl xanthine (DPCPX) as well as A_2-agonist [^{3}H]-CGS 21680 binding is affected by actions at the G-protein level. We used four guanine nucleotide derivatives (GTP, GTPγS, GPP(NH)P and GDPβS) as well as pertussis toxin (PTX) to influence the G-proteins. The effects of magnesium ions were also examined.

Thin (10 μm) sections of rat brain were mounted on slides, treated with adenosine deaminase, and incubated with radioactive ligand for 2–4 h in the presence of additions as indicated. Non-specific binding was determined with 10–20 μM R-PIA for A_1 receptors or 20 μM 2-chloroadenosine for A_2 receptors. Dried sections together with standards were apposed to [^{3}H] sensitive film. After development, the results were analyzed using an Imaging Research system. The results were evaluated using GraphPad Instat.

CGS 21680 binding, which was associated with DA-rich brain regions, was stimulated tenfold by magnesium (EC_{50} 0.5 mM), whereas the B_{max} for CHA or DPCPX was essentially unaltered. GTP, GTPγS and GPP(NH)P decreased high affinity binding at both receptors. Magnesium tended to decrease GTP potency, but this could be accounted for by a Mg-dependent hydrolysis. GDPβS displacement of the A_1 agonist was decreased by Mg. Mg decreased the rate of dissociation of agonists and in the case of CHA binding virtually no dissociation was observed over 4 h. Increases in agonist binding, including that of endogenous adenosine, to A_1 receptors were parallelled by decreases in antagonist binding. In solubilized preparations PTX was as effective as GTP in shifting CHA binding to low affinity. However, in intact brain slices PTX was virtually ineffective both when given *in vivo* and *in vitro*, despite ADP ribosylation of more than 80% of the substrate proteins. ADP ribosylation was increased when receptors were dissociated from G-proteins with GDPβS.

In conclusion, the present results demonstrate that A_1 receptors appear to be precoupled to G-proteins, whereas A_2 receptors are not. Magnesium promotes the high affinity state, being a complex with holotrimeric G-protein with no associated guanine nucleotide, of both receptors. In the case of A_1 receptors, this high affinity state binds endogenous adenosine so tightly that the receptor number is spuriously underestimated. Furthermore, the high affinity conformation of the receptor, stabilized by endogenous or exogenous agonist, prevents PTX from ADP ribosylating the associated G-protein.

Acknowledgements: This work was supported by the Swedish Medical Research Council (2553) and the Promotion Fund of the International Coffee Organization. F.E.P. is a Medical Research Council of Canada/Ciby-Geigy Fellow.

312P NEURONAL P_2-PURINERGIC (OR NUCLEOTIDE) RECEPTORS: ACTION OF ADENOSINE 5'-O-(3-THIOTRIPHOSPHATE) ON PC12 CELLS

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While ATP is widely accepted as a neurotransmitter at neuroeffector junctions (e.g. neuron/muscle synapse) evidence relating to neuron-neuron P_2-purinergic transmission is sparse. In this context the identification and characterisation of receptors for ATP/ADP on neurons is of central importance. Here we partially characterise the phospholipase C mediated response of a widely used neuronal model, the rat phaeochromocytoma derived PC12 cell line, to the stable ATP analogue adenosine 5'-O-(3-thiotriphosphate) (ATPγS). In addition we ask whether this is by a purinergic receptor or to one which can also respond to the pyrimidine UTP (uridine 5'-trisphosphate) and which should therefore be called a 'nucleotide receptor' (see O'Connor et al, 1991).
The PC12 cells were grown to confluence in 24-well plates. For measurement of total [^{3}H]inositol phosphates they were labelled for 48h with 2 μCi/ml myo-(2-^{3}H)-inositol in M199 medium with 5% foetal calf serum. Stimulation was with agonists at 30 μM except where indicated, in a balanced salt solution with 10-30 mM LiCl. For separated inositol phosphates cells were incubated in 3.3 μCi /ml, stimulation was without LiCl, and separation was on small AG1 columns.
Incubation at 37° for 30 min with 30 μM of the following agonists gave a stimulation of total [^{3}H]inositol phosphates (percent of basal, mean ± SEM, n = 4 or more separate experiments) of: ATPγS, 307 ± 32; ATP, 175 ± 17; ADP, 130 ± 9.5; UTP, 196 ± 19. The following gave no response: 2-methylthio ATP; β,γ-methylene ATP; AMP; adenosine; UDP; UMP; uridine. The response to ATPγS was approximately linear through 40 min, EC_{50} = 7.93 ± 0.76 μM (n = 8) with little dependency on extracellular calcium. The response was inhibited by suramin. On separation of inositol phosphates it was seen that [^{3}H]Ins P_3 reached a peak at 5-10 s, while [^{3}H]Ins P_2 and Ins P_1 peaked at 60 - 120 s. [^{3}H]Ins P_4 showed a slow and sustained rise. Experiments adding both ATPγS and UTP at 30 μM showed the same total [^{3}H]inositol response as ATPγS alone. Dose-response experiments for ATPγS in the presence and absence of 30 μM UTP showed a tendency for a raised EC_{50} for ATPγS in the presence of UTP.
These results show that ATPγS acts on phospholipase C linked receptors which conform to neither P_1, P_{2x} nor P_{2y} pharmacology. This may indicate the presence of an 'atypical' P_2 receptor, as suggested previously for a different cell type (Allsup and Boarder, 1990) or of a 'nucleotide receptor' capable of recognising both purines and pyrimidines.

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313P POTENTIATION OF CYCLIC AMP MEDIATED CORTICOTROPHIN (ACTH) RELEASE BY DENBUFYLLINE *IN VIVO* AND *IN VITRO*

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Alkylxanthines such as caffeine and denbufylline stimulate the secretion of corticotrophin in the rat. Our previous studies have suggested that their effects may be mediated in part at the pituitary level and that they may involve inhibition of phosphodiesterase enzymes (PDE) and hence elevations in intracellular cyclic AMP (Hadley, Flack & Buckingham, 1990). Accordingly, we have examined the effects of denbufylline (a selective inhibitor of type IV PDE) on the resting and neurochemically-evoked secretion of ACTH in vivo, in rats in which the release of endogenous corticotrophin releasing factors (CRF's) is blocked pharmacologically (Arimura, Saito & Schally, 1967), and in vitro, from rat pituitary segments (Hadley et al, 1990). Measurements of tissue immunoreactive cyclic AMP (cAMP) content were also made in the in vitro studies. Denbufylline (0.1-2.5mg/kg, i.p.) produced pronounced, dose-dependent increases in the plasma corticosterone concentration in untreated control rats ($p<0.01$ v. saline vehicle 2.0ml/kg, i.p.). Its effects were greatly attenuated ($p<0.01$) in rats in which the endogenous release of CRFs was blocked. Both hypothalamic extact (5HE/kg, i.v.) and synthetic rat CRF-41 (2μg/kg, i.v.) stimulated ($p<0.05$ v. saline vehicle 2.0ml/kg, i.v.) the release of corticosterone in the "blocked" rats. Denbufylline potentiated the responses to the tissue extract ($p<0.05$) but did not appear to influence those to synthetic peptide. In vitro, denbufylline alone (0.01-1mM) had no discernable effects on the pituitary cAMP content but produced small but significant ($p<0.05$) increases in ACTH release. However, in these and lower concentrations (0.1-1μM) the xanthine potentiated markedly ($p<0.01$) the increases in both tissue cAMP content and ACTH release induced by submaximal concentrations of hypothalamic extracts (0.1 HE/ml) and forskolin (10μM). In contrast, it failed to enhance the changes in nucleotide content and peptide release in vitro induced by CRF-41 (10nM). The results provide further evidence that denbufylline inhibits phosphodiesterase within the pituitary gland and suggest that this action may contribute to the hypersecretion of ACTH the drug evokes in vivo. The differential effects of denbufylline on the corticotrophic responses to hypothalamic extracts and CRF-41 apparent in vivo and in vitro require further study.

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314P SELECTIVE CCK_A AND CCK_B RECEPTOR LIGANDS REVEAL TWO POPULATIONS OF CCK RECEPTORS IN THE VENTROMEDIAL HYPOTHALAMUS OF THE RAT *IN VITRO*

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Autoradiographic and binding methods have shown the majority of CCK sites detected in the rat brain to be of the CCK_B subtype (Hill *et al.*, 1987). Previous electrophysiological studies have relied on agonist selectivity to confirm the existence of functional CCK_B sites in the ventromedial hypothalamus (VMH) (Boden & Hill, 1988). The development of highly selective CCK_B antagonists (Hughes *et al.*, 1990) and the CCK_A antagonist devazapide (Chang & Lotti, 1986) has allowed us to investigate the homogeneity of the CCK receptor population in the VMH in more detail. Neurones in this region responded with an increase in firing rate to CCK application. In the present study we have used the non-selective CCK receptor agonist, the sulphated octapeptide of CCK (CCK), and the selective CCK_B receptor agonist pentagastrin (PG) together with devazapide and either of the selective CCK_B receptor antagonists CI-988 or PD136450 to characterize the CCK receptor involved. Extracellular recordings were made from single neurones in the rat VMH in vitro and analyzed as described previously (Boden & Hill, 1988; Hughes *et al.*, 1990). Most (18 of 22) of these neurones also responded to pentagastrin application. The pentagastrin response was consistently sensitive to bath application of either CI-988 or PD136450 yielding equilibrium constants of 7.46 ± 3.03nM (n=4) and 8.63 ± 1.69nM (n=3) respectively, which compare favourably with their equilibrium constants in CCK_B receptor binding assays. The sensitivity of the CCK response to CI-988 was found to be more variable and on some neurones (4 of 22) the CCK response was not blocked at all. On these neurones the residual response to CCK was blocked by 10nM devazapide yielding an equilibrium constant of 224.09pM (n=4). These results suggest that the firing rate of neurones in the VMH in an in vitro slice can be influenced by CCK acting on either CCK_B or CCK_A receptors or a mixture of both. The conditions which favour expression of either receptor type are currently under study.

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315P EFFECTS OF ENALAPRILAT ON THE RENAL ADAPTATIONS TO CONSTANT FUROSEMIDE INFUSION IN CONSCIOUS RATS

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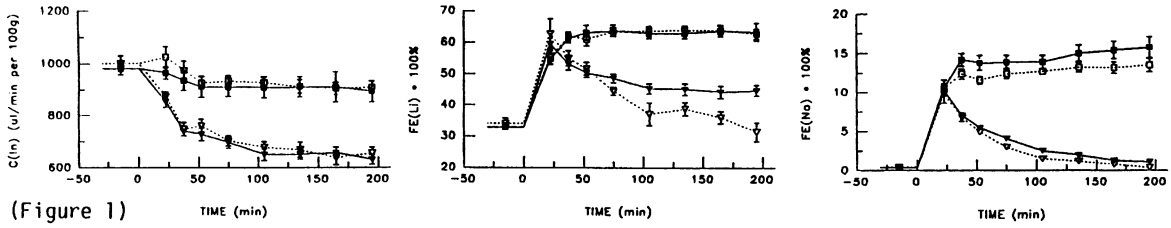
During constant administration of a diuretic, the initial volume depletion activates renal neuroendocrine mechanisms which prevent further volume losses by stimulating renal conservation of Na and water.

In this study we examined the role of the renin-angiotensin (RA) system for the natriuretic-antinatriuretic effects induced by constant i.v. infusion of furosemide (F) in rats (Christensen, S. *et al* 1986). F was infused by constant rate (1.5 mg/h) for 3 hours to conscious chronically instrumented rats with and without simultaneous volume replacement (Vol) of urinary losses by computerized infusion of 100 mM NaCl + 10 mM KCl. To block the RA-system Enalaprilat (E) was infused i.v. before and during F-infusion (100 µg bolus; 100 µg/h). Systemic and renal ACE inhibition was documented by impaired pressure response to angiotensin I and by measuring ACE activity in plasma and renal tissue. Mean arterial blood pressure (MAP) was continously recorded and clearances of lithium, [3H]-inulin and [14C]-tetraethylammonium were used as markers for the delivery of fluid from proximal tubules, the glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF), respectively.



(Figure 1)

Figure 1 shows selected parameters in four groups: F(▾) (n=10), F+Vol (■) (n=8), F+E (▽) (n=8) and F+E + Vol (□) (n=8), F being infused from time = 0 min. E did not influence the haemodynamic compensation to F infusion, i.e. the volume dependent decline in GFR to 60-70% of initial value. Furthermore E had only little influence on the characteristic time profiles of urine flow, natriuresis or fractional Na excretion FE (Na) in rats infused with F. The only significant tubular effect of E was a more fast decline in C (Li) and FE(Li) during F infusion. This was probably not due to distal lithium reabsorption induced by the progressive fall in MAP (from 105 to 90-95 mmHg) since the fall in MAP was the same in groups F+E and F+E+Vol.

It is concluded that - in contrast to the general view - the RA system has only minor importance as mediator of the acute haemodynamic and tubular adaptations to F induced volume contraction, at least in rats. In the present study blockade of the system in fact tended to enhance the neurally indicated (Petersen, J.S. *et al* in press) Na reabsorption in the proximal tubules, but this additive effect could be unspecific due to the associated fall in MAP.

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316P EFFECTS OF CONSTANT INFUSION OF DIURETICS ON 24-HOUR LITHIUM CLEARANCE IN CONSCIOUS CHRONICALLY INSTRUMENTED RATS

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During constant diuretic treatment the initial volume loss activates neuro-endocrine antinatriuretic mechanisms which limit further volume depletion by stimulating tubular sodium reabsorption. The extent and tubular site of such compensatory Na-reabsorption may depend on the type of diuretic and the degree of volume depletion, and this study used the lithium clearance (C_{Li}) technique to evaluate the involvement of the proximale tubule in diuretic stimulated Na-reabsorption during constant infusion after a loop diuretic (furosemide; F) and an early distal diuretic (bendroflumenthiazide; B). The drugs were infused intraperitonally to chronically catheterized female rats by means of implanted osmotic minipumps (F 8 mg/24 h; B 4 mg/24 h) and on the 6th day of infusion clearances of Na, Li and inulin were measured over 4 periods of 6 hours each. Control (C) and diuretic groups were subdivided into one group which received volume replacement (Vol = access to 0.9% NaCl) and one which did not. N = 8-9 animals in each subgroup.

24-hour values of GFR (C_{In}) were not different in any group. Fractional Na excretion increased from 0.5% in group C to 2.8% in group F + Vol and 0.8% in group B + Vol, but was unchanged in the diuretic groups without volume replacement. C_{Na}/C_{Li} - which expresses the urinary Na excretion as a fraction of the proximal delivery - was 1.7% in group C + Vol, 7.0% in group F + Vol, and 3.7% in group B + Vol. Fractional Li excretion (C_{Li}/C_{In}) - an estimate of the fractional rejection of Na ions from the proximal tubules - was ~28% in groups C and C + Vol, ~19% in groups B and B + Vol, ~30% in group F, and ~37% in group F + Vol.

These results indicate that chronic administration of F to rats does not lower 24h-C_{Li} like does chronic administration of a thiazide diuretic. This is in accordance with our previous study in normal human volunteers (Shalmi *et al*, 1991). On the contrary, when Na losses were prevented by free availability of saline, F treatment caused an increase of C_{Li}, like previously shown in acute experiments (Christensen, 1990).

Our findings are consistent with the hypothesis that Furosemide inhibits Na-reabsorption in the proximal tubules in additon to its well known effects on the loop of Henle. This proximal effect of F may normally be masked by some neuro-humoral compensations activated by volume depletion (Petersen *et al*, 1991), but is clearly expressed when volume losses are prevented.

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317P EFFECT OF RECOMBINANT HUMAN ERYTHROPOIETIN ON THE RENAL VASCULATURE OF THE RAT

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Recombinant human erythropoietin (rHuEpo) is used to stimulate red cell production in chronic renal failure patients who suffer from severe anaemia. It is increasingly reported that in these patients there is development of hypertension or exacerbation of pre-existing hypertension during treatment with rHuEpo. The cause of the increase in blood pressure and total peripheral resistance is unknown but could be due to a direct action of the hormone on the vasculature. This possibility was explored in the present study by examining the effect of acute and chronic administration of rHuEpo on blood flow through the cortical and papillary regions of the kidney and determining whether the hormone modified the renal vasoconstrictor responses to angiotensin II, vasopressin or phenylephrine.

Male Wistar rats, 128±2g (n=5-7), were anaesthetised with sodium pentobarbitone, 60mg/kg i.p., and a carotid artery and jugular vein cannulated for blood pressure monitoring and infusion of drugs, respectively. The left kidney was exposed and supported in a plastic cup with its dorsal surface uppermost to allow exposure of the papilla. Laser-Doppler flowmetry was used to measure perfusion in the renal cortex and papilla as previously described (Huang, Davis & Johns, 1991). Red cell flux was reported as perfusion units (PU) which have a value of 10mV. Administration of 50U/kg rHuEpo i.v. had no effect on blood pressure or renal haemodynamics whereas 150U/kg i.v. caused a significant ($P<0.01$) 6% reduction in cortical perfusion (182±16 versus 193±15 PU) while in the vehicle treated animals cortical perfusion rose significantly by 9% ($P<0.05$). In rats chronically treated with rHuEpo, 150U/kg s.c. three times per week for one week, haematocrit was increased (51.1±0.1 versus 46.8±1.1%) and both cortical and papillary perfusions were reduced (both $P<0.05$) compared to the vehicle treated animals (159±6 versus 176±8 PU for cortex and 199±6 versus 225±10 PU for papilla). Infusion of angiotensin II at 45ng/kg/min increased blood pressure by some 10% ($P<0.001$) and reduced cortical perfusion by 14% ($P<0.01$) but had no effect on papillary perfusion, whereas vasopressin, 20mU/kg/min, increased blood pressure by 35-40% ($P<0.001$) and reduced cortical and papillary perfusions by 13 and 19 %, respectively ($P<0.001$) while phenylephrine, given at 20μg/kg/min, increased blood pressure by 20% ($P<0.01$) and decreased cortical and papillary perfusions by 14 and 16%, respectively (both $P<0.01$).The magnitudes and patterns of these vasoconstrictor agents were not affected by either the acute or chronic treatment with rHuEpo.

These results show that therapeutic doses of rHuEpo can acutely cause a small vasoconstriction in the cortex while chronic administration induces slightly larger reductions in perfusion of both cortex and papilla. However, the renal vascular responsiveness to a range of vasoactive substances was unaltered. These reductions in renal perfusion in the presence of the hormone implies increased resistance within the kidney which could contribute to the raised total peripheral resistance observed during administration in man.

Huang, C, Davis, G & Johns E.J. (1991) Exp .Physiol .76, 399-408.

318P CHARACTERISTICS OF α-ADRENOCEPTORS IN THE RAT RENAL VASCULATURE

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There is increasing evidence that the contraction of vascular smooth muscle through the activation of α_1-adrenoceptors is caused either by the release of calcium from intracellular stores via the phosphotidyl inositol pathway or the influx of extracellular calcium (Minneman, 1988). The present study was undertaken in anaesthetised (sodium pentobarbitol, 60 mg/kg i.p.) male Wistar rats, 280-310 g, to assess, in vivo, the post-receptor mechanism utilised by the α_1-adrenoceptors in the renal vasculature. Briefly, following tracheostomy, a carotid artery and jugular vein were cannulated for the measurement of blood pressure and the infusion of saline. The left kidney was exposed via mid-line abdominal incision and an electromagnetic flowmeter probe fitted on the renal artery. The renal nerves were isolated and dissected to give a sufficient length for stimulation. The ileac artery was cannulated such that the tip of the cannula lay at the level of the renal artery to allow close arterial administration of all drugs. The renal vasoconstrictor responses to renal nerve stimulation (1,2,4,6,8,10 Hz at 15 v and 0.2 ms), phenylephrine (10,20,30,40 μg) and methoxamine (20,40,60,80 μg) were determined before and after the administration of amlodipine (200 μg kg^{-1} bolus followed by 50 μg $kg^{-1}h^{-1}$ and twice this dose), chloroethylclonidine (CEC) and 5-methylurapidil (meU) at 5 μg kg^{-1} followed by 1.25 μg $kg^{-1}h^{-1}$ and twice this dose in 3 different groups of animals. The rate of infusion was fixed to deliver 6 ml h^{-1}.

		Mean change in renal blood flow (ml/min) ± s.e.mean		
Group	Treatments	Renal nerve stimulation	Phenylephrine	Methoxamine
A	Control	1.59 ± 0.40	2.41 ± 0.44	1.88 ± 0.41
	200 μg kg^{-1} amlodipine	1.08 ± 0.27*	1.63 ± 0.36*	1.49 ± 0.29*
	400 μg kg^{-1} amlodipine	0.92 ± 0.22*	1.53 ± 0.30*	1.30 ± 0.29*
B	Control	1.60 ± 0.41	3.19 ± 0.47	1.99 ± 0.61
	5 μg kg^{-1} CEC	1.48 ± 0.30	3.16 ± 0.55	1.79 ± 0.51
	10 μg kg^{-1} CEC	1.27 ± 0.32*	3.11 ± 0.55	1.85 ± 0.53
C	Control	2.37 ± 0.51	2.92 ± 0.58	2.34 ± 0.61
	5 μg kg^{-1} 5-meU	1.64 ± 0.36*	1.51 ± 0.27*	1.03 ± 0.28*
	10 μg kg^{-1} 5-meU	1.55 ± 0.35*	1.19 ± 0.18*	0.80 ± 0.23*

* = $P<0.05$ (ANOVA), $n \geq 7$

The findings of the present study are consistent with the suggestion that the receptors found in the Wistar rat renal vasculature are predominantly those dependent on extracellular calcium, ie α_{1a}-subtype as proposed by Minneman (1988) and this is further supported by the pattern of attenuation of responses obtained in the group treated with 5-methylurapidil which has been proposed to be an α_{1a}-selective antagonist.

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319P EFFECTS OF RO 31-5460 ON RENAL ISCHAEMIA AND REPERFUSION DAMAGE IN RATS

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Ro 31-5460 (4-hydroxy-9H-pyrido[3,4-b]indole-1-carboxaldehyde) is a naturally occurring compound found in the bark of the Chinese tree Picrasma quassioides. Previous studies have shown Ro 31-5460 to be a potent xanthine oxidase (XO) inhibitor which is cardioprotective against reperfusion injury following myocardial ischaemia, in both rabbits and dogs (Lad et al. this meeting). In this study, we have determined the effectiveness of Ro 31-5460 pretreatment on renal damage (assessed by the determination of plasma urea and creatinine concentrations), following renal ischaemia and subsequent reperfusion. The method used in this study was based on that of Paller et al. (1984). Male rats were anaesthetised, their right kidneys were removed and left kidneys exteriorised. Heparin was administered intravenously (50 IU/kg) followed by saline or the drug under test. After ten minutes the left renal artery, vein and ureter of each animal were totally occluded with clips. During this period the animals were kept on, and covered by, warming blankets (37°C) and the ischaemic kidneys were kept moist with saline-soaked swabs. The clips were removed after 45 min when the animals were sutured and allowed to recover. Arterial blood samples were taken under ether anaesthesia from the tail 1h prior to surgery and at 24, 48 and 72h post-reperfusion. Plasma creatinine and urea concentrations were then determined from these samples using a 'Cobas Bio' analyser.

Initial studies showed that 45 min ischaemia produced large increases in plasma urea (37.6 ± 1.6 mg/dl at 0h, to 349.0 ± 82.3 mg/dl at 72h) and creatinine levels (62.5 ± 2.1 mol/ml at 0h, to 362.6 ± 45.2 mol/ml at 48h). Ro 31-5460 pretreatment at both 0.3 mg/kg and 1 mg/kg, i.v. 10 min prior to ischaemia and 5 min prior to reperfusion (double-dosing) significantly ($P<0.05$) reduced elevated plasma urea and creatinine levels. Similar double-dosing pretreatment with the free radical scavenger 1,3-dimethyl-2-thiourea (DMTU) at 1 and 10 mg/kg also significantly reduced the elevated plasma urea and creatinine concentrations. In contrast, the reference XO inhibitor allopurinol (40 mg/kg, i.v., 10 min prior to ischaemia) failed to prevent the elevation of both urea and creatinine in the plasma.

During these initial studies, there were indications that animals which received Ro 31-5460 had a higher survival rate. Specific experiments were therefore carried out using a 1h period of renal ischaemia prior to reperfusion. This produced a high mortality in the control group so any significant reduction in deaths by drug pretreatment could be demonstrated. Blood samples were not taken in these studies in case the sampling procedure under ether anaesthesia contributed to mortality. Control results showed mortalities of 0% (24h), 68% (48h) and 76% (72h). Pretreatment with either allopurinol (20mg/kg, i.v. - double-dosing) or DMTU (1 mg/kg, i.v. - double-dosing) failed to reduce deaths but similar treatment with Ro 31-5460 at 0.1 mg/kg, i.v. (32% at 48h and 48% at 72h), and 0.3 mg/kg, i.v., (48% at 72h) significantly reduced mortality.

It is concluded that Ro 31-5460 appears useful in the prevention of reperfusion damage following renal ischaemia, although its mode of action is unclear.

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320P MECHANISM OF ANTIFIBRILLATORY ACTION OF UK-66914: STUDIES IN RABBIT

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UK-66914 is a new antiarrhythmic which prolongs QT interval and suppresses ventricular arrhythmias in dog. It is believed to act via selective blockade of delayed rectifier K^+ current, (IK; Gwilt et al., 1991). In the present study we examined the effects of UK-66914 on QT interval and incidence of ventricular fibrillation (VF) in rabbit.

Rabbit hearts (n=13/group) were perfused with solution containing (in mM) NaCl 118.5, $NaHCO_3$ 25.0, KCl 3, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 2.8 and glucose 11.1, pH 7.4, 37°C for 5 min, then randomized to solution containing 0, 0.3 or 1μM UK-66914. Left regional ischaemia was induced 5 min later by coronary ligation and was maintained for 30 min. Ischaemia was followed by 5 min of reperfusion. QT interval was measured 1 min before randomization (pre-drug), 1 min before ischaemia (Isch. -1 min) and 15 min after the onset of ischaemia (Isch. +15 min), and was corrected for heart rate (QTc=QT/RR).

Table 1.

	Incidence of VF		QTc		
	Ischaemia	Reperfusion	Pre-drug	Isch. -1 min	Isch. +15 min
Control	2/13	10/13	0.57±0.02	0.59±0.03	0.58±0.03
0.3 μM	1/13	5/13*	0.59±0.02	0.64±0.03	0.67±0.02*
1 μM	3/13	4/13*	0.60±0.02	0.68±0.03*	0.68±0.03*

UK-66914 (0.3 and 1 μM) decreased the incidence of reperfusion-induced VF and prolonged QTc (*$p<0.05$). Detection of any antifibrillatory action during ischaemia was not possible owing to the low incidence of VF in control hearts; there were no proarrhythmic effects. Previous findings (Rees & Curtis, 1991) showed that UK-66914 does not suppress VF or prolong QT in rat, a species deficient in cardiac IK (Tande et al., 1990), whereas present findings show that UK-66914 does suppress VF and prolong QT in rabbit, a species which does possess cardiac IK (Colatsky et al., 1990). Thus, IK blockade and QT prolongation appear to be sufficient and necessary for suppression of VF by UK-66914.

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321P ABANOQUIL: A NOVEL α_{1A}-ADRENOCEPTOR ANTAGONIST

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Abanoquil, UK-52,046, (4-amino-6,7-dimethoxy-2-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl) quinoline methanesulphonate) is a novel α_1-adrenoceptor antagonist which displays antidysrhythmic activity at doses not producing haemodynamic effects (Aubrey *et al*, 1988). We report here its activity on the α_1-adrenoceptor subtypes originally identified by Morrow and Creese (1986) and designated α_{1A}- and α_{1B}- adrenoceptors. It has recently been suggested that UK-52,046 is able to distinguish between α_1-adrenoceptors *in vitro* (Mir and Fozard, 1991).

In our studies, [3H]-prazosin was used as the radioligand as it is equipotent at α_{1A}- and α_{1B}-adrenoceptors. Rat liver membranes (α_{1B}:α_{1A} 90:10) were used to assess α_{1B} activity while rat heart membranes (α_{1A}:α_{1B} 60:40) were used to assess α_{1A} activity (after preincubation with chlorethyl clonidine, (CEC) to remove α_{1B}). Binding assays were conducted at 25°C for 30min in 1ml microtitre blocks containing 200µg protein, 0.2nM [3H]-prazosin and buffer/displacer in a total assay volume of 500ul. Non-specific binding was determined in the presence of 10µM phentolamine for all assays. Results are summarized in Table 1. Prazosin and doxazosin were equipotent at α_{1A} and α_{1B} sites. WB4101, phentolamine and 5-methyl urapidil, while exibiting varying potencies, clearly demonstrate moderate α_{1A} selectivity. UK-52,046 was the most potent and selective α_{1A} compound tested, being approximately 100-fold more potent at α_{1A} than α_{1B} sites. UK-52,046 displays little or no activity at other monoamine binding sites, or several other receptor sites.

Recent work (Balzo *et al*, 1990) suggests that the α_{1B}-adrenoceptor in cardiac tissue may be linked to a decrease in automaticity while the α_{1A}-adrenoceptor may be linked to an increase in automaticity. Thus highly selective blockade of α_{1A} -adrenoceptors could contribute to the antiarrhythmic activity seen with UK-52,046.

TABLE 1

	IC_{50}nM, Mean, ± SEM					IC_{50}nM, Mean, ± SEM			
Compound	α_{1A}	n	α_{1B}	n	Compound	α_{1A}	n	α_{1B}	n
Prazosin	0.51±0.05	4	0.65±0.21	4	WB4101	0.15±0.02	5	27±8	5
Doxazosin	8.15±1.2	4	5.80±0.75	4	5-Me-urapidil	5.10±1.1	3	526±124	3
Phentolamine	5.80±0.95	4	38±10	4	UK-52,046	0.003±0.001	6	0.27±0.04	6

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322P REDUCTION OF REPERFUSION-INDUCED MYOCARDIAL TISSUE NECROSIS BY RO 31-5460 IN ANAESTHETISED RABBITS AND DOGS.

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Early reperfusion of ischaemic myocardium may have beneficial and deleterious effects on reversibly injured cells (Braunwald & Kloner, 1985). With the advent of thrombolytic therapy, there is a need for agents that can be given simultaneously with a thrombolytic agent to reduce reperfusion injury. In the present study, we have investigated the effects of a novel agent, Ro 31-5460 (4-hydroxy-9H-pyrido[3,4-b]indole-1-carboxaldehyde) on myocardial tissue necrosis in rabbit and dog. In vitro studies showed that Ro 31-5460 inhibited rat cardiac xanthine oxidase (XO) (IC_{50} 0.7 nM). Therefore, we compared the effects of Ro 31-5460 with the XO inhibitor, allopurinol, in dogs (high cardiac XO model) and its active metabolite, oxypurinol, in rabbits (low cardiac XO model).

Male rabbits (2.5-3.5 kg) were anaesthetised, ventilated and the coronary artery occluded (CAO, 30min) and reperfused (Coker, 1989). After 2h of reperfusion, 66 ± 6% of the risk area was infarcted (assessed by nitro-blue tetrazolium staining, NBT) in the vehicle-treated group (n=8). Pre-treatment with Ro 31-5460 (1 mg/kg, i.v., 10min prior to CAO) reduced the area of infarcted tissue (38 ± 5%, n=8, P<0.05). Additionally, administration of Ro 31-5460 after the onset of ischaemia (5min prior to reperfusion) reduced the infarct/risk zone ratio at 0.1 and 0.3 mg/kg (37 ± 8% and 36 ± 8%, respectively, n=8), compared to the vehicle-treated group (59 ± 5%, n=8, P<0.05). Oxypurinol (50 mg/kg, i.v., given 10min prior to CAO) afforded no significant protection against reperfusion-induced tissue necrosis (57 ± 8%, compared to 60 ± 6% in the control group, n=8).

Male greyhounds (26-32 kg) were anaesthetised, ventilated and prepared for open-chest coronary occlusion, infarct size determination and collateral blood flow measurement (Uraizee et al., 1987; Kingma et al., 1988). Infarct size as a result of CAO (90min) followed by reperfusion (3h) was related to the anatomical risk zone size and epicardial collateral blood flow (ECBF). Infarct size was inversely related to ECBF in both control and drug-treated groups. Analysis of covariance showed that the relation between infarct size and ECBF in the Ro 31-5460-treated group (0.3 mg/kg, i.v., given 10min prior to CAO and further 5min prior to reperfusion) differed significantly from the vehicle group (F=18.1, P<0.001). Thus, for a given ECBF, the resultant infarct size in the Ro 31-5460-treated group was significantly reduced compared to control. Allopurinol pre-treatment (400 mg, p.o., 24h prior to surgery and further 50 mg/kg, i.v.,given 10 min prior to CAO, n=5) resulted in no clear reduction in myocardial infarct size for a given ECBF. No significant differences in the areas at risk and the haemodynamic parameters were observed between the vehicle- and drug-treated groups in the studies above.

Pre-treatment with Ro 31-5460 reduced the extent of reperfusion-induced tissue necrosis in both rabbit and dog models, in contrast to allopurinol and oxypurinol. Thus, despite its activity as a XO inhibitor, the mechanism of Ro 31-5460 is presently unclear.

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323P ISCHAEMIA AND REPERFUSION INJURY POTENTIATES THE VASOCONSTRICTOR EFFECTS OF ENDOTHELIN-1 IN THE ISOLATED PERFUSED HEART OF THE RAT

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Plasma levels of ET-1 (and its precursor bigET) are elevated in patients with acute myocardial infarction *(Miyauchi et al., 1989)*. Cardiac membranes obtained from isolated perfused hearts of rats subjected to 30 min cessation of flow followed by reperfusion showed an increased density of ET-1 binding sites *(Liu et al., 1990)*. Most importantly, monoclonal antibodies to ET-1 prevent the vascular effects of the peptide and reduce infarct size after coronary artery ligation and reperfusion in anaesthetized rats *(Watanabe et al., 1990)*. Here we investigate the effects of ischaemia and reperfusion on the vasoconstrictor effects of ET-1 in the rat isolated perfused heart.

Male Wistar rats (body weight: 350-450g) were anaesthetized with sodium pentobarbitone (75 mg kg^{-1} i.p.). After injection of heparin (500 IU i.v.) and thoracotomy, the hearts were excised and perfused (according to Langendorff) at a constant flow of 10 ml min^{-1} with oxygenated (95% O_2, 5% CO_2) Krebs' solution. Changes in coronary perfusion pressure (CPP) and isometric force of contraction (resting tension of 1g) were recorded in control hearts and hearts subjected to 30 min of cessation of flow (MI) followed by 30 min of reperfusion. Drugs were given as bolus injections at 10 min after restoration of flow (post-ischaemia). Statistical comparisons between groups were made by two-way ANOVA.

On reperfusion of hearts subjected to MI, immediate CPP was 82±2% of resting CPP and then gradually rose to 120±7% at the end of the experiments (n=9). This rise in CPP was not significantly affected by indomethacin (INDO, 5.6 μM; 141±11%; n=4). In non-ischaemic control hearts, ET-1 (100 pmol) produced an increase in CPP (peak at 1 min: 187±17%; n=4), which returned to basal values within 5 min. Although cyclooxygenase inhibition had no effect on the peak response to ET-1 (179±13%; n=7), INDO substantially prolonged the duration of the pressor response throughout the experiment ($p<0.05$ at 5-20 min). Similarly, MI did not potentiate the peak increase in CPP produced by ET-1 at 1 min, but prolonged the pressor effect throughout the reperfusion period. For instance, the CPP at 20 min after ET-1 was 127±13% in the control hearts and 169±9% in hearts subjected to MI ($p<0.05$). Moreover, INDO further potentiated the rise in CPP induced by ET-1 at all measured time points from 1-20 min. For instance at 5 min the rise in CPP produced by ET-1 after MI was 143±8% in the absence and 205±21% in the presence of INDO (n=8-9, $p<0.05$). When compared to ET-1, phenylephrine (PE, 1 μmole; n=4) or the thromboxane A_2-analogue U46619 (3 nmoles; n=6) produced a similar rise in CPP in non-ischaemic control hearts. However, the pressor responses to PE and U46619 were neither potentiated nor prolonged by MI and reperfusion (n=5).

Thus, the coronary vasoconstrictor responses to ET-1, but not to PE or U46619, are significantly prolonged in this model of myocardial ischaemia, an effect which may depend on an ischaemia-induced impairment of the release of prostacyclin. These findings support the hypothesis that enhanced levels of ET-1 may play a role in the extension of ischaemia and reperfusion related injury of the heart. ***(This work was supported by the Parke-Davis Pharmaceutical Research Division of Warner-Lambert Co.)***.

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324P EFFECT ON CARDIAC CONTRACTILE PATTERN OF EARLY AND LATE ANGIOTENSIN CONVERTING ENZYME INHIBITION AFTER MYOCARDIAL INFARCTION IN RATS

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Compensatory hypertrophy has been demonstrated in the surviving myocardium after ligation of the left coronary artery in animals (Anversa et al, 1990) and after acute myocardial infarction in patients (Ginzton et al, 1989). Preliminary animal studies suggest that angiotensin converting enzyme (ACE) inhibitors may inhibit the hypertrophy brought about by left coronary ligation (Pfeffer et al, 1985). The functional significance of the hypertrophy has not been clarified.
The purpose of this study was to examine the effect of treatment (initiated early or late) with ACE inhibitors on the physiological characteristics of contraction and relaxation (basal and during isoprenaline stimulation) of surviving myocardium in rats after ligation of the left coronary artery.

Wistar rats were treated with placebo (INFARCTED) or perindopril 2 mg/kg/day starting the day after (PER. EARLY) or starting 14 days (PER. LATE) after left coronary artery ligation (LCAL). Sham-operated (SHAM) rats served as controls. Four weeks later isometrically contracting and field stimulated (1 Hz) papillary muscles from the right ventricle were used to determine: Maximum active developed tension (DT,mN), time to maximum active developed tension (t_{DT},ms), time to 50 % decline from maximum active developed tension ($t_{1/2}$,ms) in control period (ctrl) and in the presence of isoprenaline 10^{-5} M (iso).
Computer sampled and analyzed data are mean ± S.E.M.

GROUP	SHAM		INFARCTED		PER. EARLY		PER.LATE	
(number)	ctrl(21)	iso(8)	ctrl(20)	iso(6)	ctrl(23)	iso(10)	ctrl(19)	iso(7)
DT (mN)	7±1	7±1	9±1*	15±1*	6±1+	10±1+	6±1+	9±1+
t_{DT} (ms)	78±2	58±1	88±3*	71±3*	81±2+	62±1*+	80±1+	62±3
$t_{1/2}$ (ms)	43±2	29±1	51±2*	37±1*	43±1+	34±1*	44±1+	33±2

* $p < 0.05$ versus SHAM. + $p < 0.05$ versus INFARCTED. Student's *t*-test
Papillary muscles after LCAL showed prolonged contraction and relaxation phases and increased maximum active developed tension in control period and during isoprenaline stimulation. Early as well as late angiotensin converting enzyme inhibition significantly limited these alterations during basal conditions. During isoprenaline stimulation early treatment significantly reduced DT and t_{DT} while late treatment only reduced DT.

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325P ENDOTOXIN AND CYTOKINES INDUCE A Ca^{2+}-INDEPENDENT NITRIC OXIDE SYNTHASE IN MYOCARDIUM AND ISOLATED MYOCYTES

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Synthesis of NO by a constitutive, Ca^{2+}-dependent NO synthase in the endocardium (Schulz et al., 1991) or treatment with sodium nitroprusside causes a negative inotropic response of cardiac muscle by increasing the level of cGMP (Smith et al., 1991). Depression of myocardial contractility is a characteristic pathophysiological event in septic shock (Solis & Downing, 1966; Gunteroth et al., 1982; Suffredini et al., 1989). Treatment with endotoxin causes the induction of a Ca^{2+}-independent NO synthase in many cells and tissues (see Moncada et al., 1991 for review). We have investigated whether the myocardium possesses the constitutive NO synthase and whether it can express the inducible enzyme. Nitric oxide synthesis, determined as the formation of ^{14}C-citrulline from ^{14}C-arginine (Knowles et al., 1990), was measured in cytosols from the left ventricular wall from rats treated with endotoxin (4 mg kg^{-1}, i.p.), or from freshly isolated cardiac myocytes from adult rats stimulated with cytokines. Cytosols from the ventricle of saline-treated control animals showed only Ca^{2+}-dependent NO synthase activity (1.30 ± 0.16 pmol min^{-1} mg^{-1} protein, n=6). After treatment with endotoxin (0.5 to 24 h) the expression of an inducible, Ca^{2+}-independent NO synthase was observed with maximal activity at 6 h (5.15 ± 1.00 pmol min^{-1} mg^{-1} protein, n=5) and which returned to baseline by 18 h. No change in the Ca^{2+}-dependent activity was observed over this time course ($n \geq 5$ for each time point). Parallel to the enzyme induction there was an increase in myocardial cGMP levels (1.23 ± 0.12, n=6 compared with 0.82 ± 0.03 pmol mg^{-1} protein, n=4; $P < 0.05$) and in the levels of plasma nitrite and nitrate (NO_x^-) (362 ± 39, n=6 compared with 23 ± 4 μM, n=7; $P < 0.05$) measured at 6 h. All these changes were prevented by pretreatment with dexamethasone (1 mg kg^{-1}). Isolated cardiac myocytes possessed Ca^{2+}-dependent NO synthase activity (4.73 ± 0.99 pmol min^{-1} mg^{-1} protein, n=4) and expressed, after 24 h treatment with tumour necrosis factor-α (20 ng ml^{-1}) and interleukin-1ß (5 ng ml^{-1}), a Ca^{2+}-independent NO synthase (4.25 ± 0.78 pmol min^{-1} mg^{-1} protein, n=4), the induction of which was prevented by incubation with dexamethasone (3 μM, n=3) or cycloheximide (10 μM, n=4). The activity of the Ca^{2+}-dependent enzyme was unaltered by any of these treatments (n=3-5 in each group). The cardiac depression which occurs during endotoxin shock, anti-tumour cytokine therapy and other conditions whose pathogenesis is linked to the release of cytokines may be due to the enhanced production of NO in the myocardium by an inducible Ca^{2+}-independent enzyme. The role of the constitutive NO synthase in myocardium is unknown, however, it may play a role in the physiological regulation of myocardial contractility.

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by naltrindole in 25 day old rats

326P DIFFERENCES IN SENSITIVITY OF ANTINOCICEPTIVE EFFECTS OF SELECTIVE δ-OPIOID AGONISTS TO ANTAGONISM BY NALTRINDOLE IN 25-DAY-OLD RATS

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The synthesis of stable non-peptide antagonists at the δ-opioid receptor (Portoghese *et al*, 1988) has allowed further investigation into the physiological role of this receptor. Recent *in vivo* studies in mice with naltrindole and its benzofuran derivative have suggested the existence of δ-receptor subtypes (Sofuoglu *et al*, 1991). We have further addressed this possibility by studying naltrindole antagonism of antinociceptive effects of selective δ-agonists in postweanling rats. Tail immersion latencies (50°C) were determined in 25 day old Wistar albino rats (60-90g), at 2, 5, 10 and 15 min. after i.p. injection of[D-Pen^2, D-Pen^5] enkephalin (DPDPE), [D-Ser^2, Leu^5, Thr^6] enkephalin (DSLET) or [D-Ala^2] Deltorphin I (DAD). For antagonism studies naltrindole was injected 10 min. prior to administration of δ-agonists. DPDPE, DSLET and DAD caused dose related increases in response latency with peak antinociception at 5 min. The ED_{75} of each of the δ-agonists was chosen for antagonism studies with naltrindole. Using these doses the peak response latencies after naltrindole treatment are shown in Table 1.

Table 1. Effect of naltrindole on peak antinociceptive responses to δ-agonists in 25 days old rats

	Tail immersion latencies (s)			
Naltrindole (mg/kg)	0	0.01	0.1	1.0
DPDPE (0.66 mg/kg)	4.9 ± 0.14	4.0 ± 0.45**	3.0 ± 0.2**	2.6 ± 0.2**
DSLET (0.65 mg/kg)	4.7 ± 0.35	3.6 ± 0.23**	2.5 ± 0.2**	2.8 ± 0.2**
DAD (0.032 mg/kg)	4.8 ± 0.37	4.4 ± 0.36	4.9 ± 0.5	2.7 ± 0.1**

Values are mean ± s.e. mean of 5-8 determinations. Saline control latencies ranged from 2.5 - 2.8 s. ANOVA of full-time response curves** $P < 0.01$ vs agonist alone.

Naltrindole (1 mg/kg) completely reversed the effects of all δ-agonists without affecting antinociception induced by the μ-agonist alfentanil. 0.1 mg/kg naltrindole abolished DSLET and DPDPE antinociception but had no effect on DAD antinociception. 0.01 mg/kg naltrindole partially attenuated responses to DSLET and DPDPE and again had less effect on responses to DAD. The differential sensitivity of these δ-agonists to antagonism by naltrindole supports the work of Sofuoglu *et al* (1991) in mice suggesting δ-receptor heterogeneity. Additionally this work suggests that DAD exerts its antinociceptive effects at a different subtype to DPDPE or DSLET.

T.J. Crook is a SERC CASE student with MSD.

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327P MORPHINE-INDUCED DEPRESSION OF PERIPHERAL NOCICEPTOR ACTIVITY FOLLOWING INFLAMMATORY HYPERALGESIA: STUDIES IN AN ISOLATED SKIN/NERVE PREPARATION OF ADULT RATS

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Primary hyperalgesia, following an inflammatory injury, is associated with changes in excitability of peripheral nociceptors and the appearance of sensitivity to peripheral opioids (Joris et al 1990). In order to study hyperalgesic phenomena in greater detail we have used an isolated skin-nerve preparation in which substances can be applied directly to the receptive field of nociceptors (Reeh, 1986)

Male rats were anaesthetised with pentobarbital (40-50 mg/kg ip) and the hind paw skin, together with the saphenous nerve, were removed. The skin was mounted, outside down, in a chamber and superfused with a physiological salt solution at 32°C, equilibrated with 95% O_2/ 5% CO_2. The saphenous nerve was partitioned into another chamber where fine fibres were dissected until single unit activity could be recorded by a silver wire electrode. Receptive fields were searched by probing the corium side of the skin with a glass rod. Electrical stimulation was used to determine fibre conduction velocity and mechanical threshold was determined using von Frey hairs. Polymodal nociceptors were identified by their sensitivity to heat (50°C) and high intensity mechanical stimulation. Following this a hollow metal cylinder was placed on the receptive field to allow the localized application of chemical solutions.
In preparations from untreated animals, capsaicin administered for 30 sec to quiescent polymodal nociceptors produced a concentration related activation. A submaximal concentration of 1µM evoked a mean 26.7±6.6 spikes/min (n=14). Morphine (10-20µM) did not affect quiescent nociceptors or nociceptors activated by 50 mM KCl stimulation. In preparations taken from animals 5 days after hyperalgesia had been induced by irradiation with u/v, a significantly greater number of spontaneously active nociceptors were recorded. In u/v treated animals responses of quiescent nociceptors to capsaicin (1 µM) were significantly enhanced (50±5 spikes/min,n=8). Morphine produced a concentration related depression of spontaneously active nociceptors from u/v (n=18) pretreated animals. This effect was prevented or reversed by 1 or 2 µM naloxone.
These data indicate that hyperalgesia is associated with changes in the excitability of peripheral nociceptors and an increase in their responsiveness to capsaicin and morphine.

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328P EFFECTS OF BRADYKININ AND PROSTAGLANDINS ON CGRP RELEASE FROM SPINAL CORD SLICES

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Bradykynin (BK) is an inflammatory mediator with a wide range of actions. As well as exciting peripheral nociceptors (Dray et al, 1989), it also depolarises primary afferent terminals in the dorsal horn (Dunn & Rang, 1990), where BK receptors are found (Steranka et al, 1988). The functional consequences of this are unknown, so we have investigated whether BK can elicit neuropeptide release from the spinal cord, or modulate the release evoked by dorsal root stimulation. The preparation used was a dorsal slice, with dorsal roots attached, cut with a vibratome from the lumbar region of the spinal cord of 20-day rats. The preparation was mounted in a perfusion chamber, with the dorsal roots (3-4 on each side) laid across platinum stimulating electrodes. The chamber was perfused with Krebs' solution (1 ml/min) at room temperature, and 5-min fractions were collected for measurement of CGRP-like immunoreactivity by radioimmunoassay. The basal release of CGRP was measured, then the dorsal roots were stimulated (10 Hz, 20V, 1 msec pulses) for 5-min. The release evoked (usually about 10-times basal) was expressed as S1. The test compound was then added to the perfusion medium, and 20 minutes later the stimulation was repeated (S2). The effect of the test drug was expressed as the ratio S2/S1. Values given are MEAN ± SEM (N).

Under control conditions, S2/S1 was 0.82 ± 0.04 (7). Addition of BK (1 µM) before S2 produced little or no release of CGRP, but significantly ($P<0.05$) increased S2/S1 to 1.65 ± 0.41 (3), showing that it enhanced the evoked release. Addition of indomethacin (1 µM) throughout the experiment had no effect on S2/S1 (0.80 ± 0.22 (3)), but in the presence of indomethacin, BK (1 µM) significantly *inhibited* the neurally-evoked release. (S2/S1 = 0.34 ± 0.02 (3)). This suggested that the enhancement produced by BK might be due to release of prostanoids (see Lembeck et al, 1976), so we tested the effects of various prostaglandins. PGE1 (0.1 -10 µM) significantly increased S2/S1 (2.25 ± 0.36 (3) at 1 µM), but PGE2 and PGI2 were ineffective up to 10 µM.

These results suggest that BK may act directly on afferent nerve terminals in the dorsal horn to inhibit neurally-evoked CGRP release, but indirectly, through prostanoid formation, to enhance release, with the latter effect predominating in the absence of cyclooxygenase inhibition. In the absence of electrical stimulation, BK did not elicit a significant release of CGRP. The nature of the BK receptors responsible for these effects on evoked release, and the transduction mechanisms involved, remain to be studied.

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329P THE EFFECTS OF SYMPATHOMIMETIC AGENTS ON THE DISPOSITION OF CODEINE AND MORPHINE IN MICE

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Increasing non-medical use of cough and cold "remedies" is a subject of growing concern in Hong Kong as we have observed that proprietary preparations containing ephedrine (EPH) and codeine are popularly abused by teenagers (Chan et al, 1990). We have shown that EPH and phenylpropanolamine (PPA) potentiate the acute antinociceptive effects of morphine and codeine in naive mice (Dambisya et al, 1990), enhance the development of tolerance and have no effect on that of physical dependence (Dambisya et al, 1991). The effects of EPH and PPA on the disposition of morphine and codeine are now reported.

Male ICR mice 30 to 35 g were used. EPH or PPA (10 mg/kg) was administered intraperitoneally 10 min prior to subcutaneous morphine (8 mg/kg) or codeine (60 mg/kg). The mice were then sacrificed at 15 and 30 min, and at 1, 2 and 3 h under light ether anaesthesia by exanguination and/or decapitation. After exanguination, blood from 3 animals was pooled and centrifuged at 2000 g for 30 min. The brain was removed immediately and later homogenized in toto and centrifuged as above. Plasma (1 ml) and all the supernatant of the homogenate were assayed for the opioids using the procedure described by Svensson et al (1982), which involved solid phase extraction using Sep-Pak C_{18} cartridges (Waters) and ion-pair reversed phase HPLC. Detection was with u.v. detector (for codeine) and coulometric electrochemical detector (for morphine) coupled in series.

EPH or PPA pretreatment did not interfere with the plasma disposition of morphine or codeine as evidenced by overlapping curves and lack of significant differences in the areas under concentration-time curve (AUC). The elimination half life was not affected by the sympathomimetics. The brain opioid levels were also apparently unaffected by EPH or PPA pretreatment. The relative ratios of brain to plasma concentrations at the time corresponding to the respective peak antinociceptive activity (15 min for codeine and 30 min for morphine, Dambisya et al, 1990) were not significantly different. Morphine was detectable around 30 min in plasma of mice that had received codeine. The ratios of AUC morphine to the corresponding AUC codeine in these groups also showed no significant differences. It is concluded that single doses of EPH and PPA do not affect the disposition of morphine and codeine in mice.

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330P EVIDENCE TO SUGGEST THAT THE ANTINOCICEPTIVE ACTION OF INTRATHECALLY APPLIED 5-HT IN THE RAT SPINAL CORD IS MEDIATED BY NON-5-HT$_3$ RECEPTORS

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Several 5HT receptor subtypes are involved in spinal 5HT antinociception. Recent studies have particularly focused on 5HT1 (El-Yassir et. al. 1990) and 5HT3 receptors (Glaum et. al. 1990). Glaum reported that in behavioural tests a 5HT3 receptor antagonist could reverse the antinociceptive action of both 5HT and a 5HT3 receptor agonist. To further investigate the role of 5HT3 receptors in spinal antinociception we have examined the effects of intrathecally applied agents in a modified in vivo electrophysiological recording preparation.

Male Wistar rats were anaesthetised with halothane. Responses to peripheral noxious thermal stimulations were recorded from dorsal horn neurones with a tungsten electrode inserted through a small slit in the otherwise intact dura. Following three control nociceptive responses 5µl of a drug dissolved in saline was injected intrathecally in the region of the lumbar enlargement. Drugs used were either 250nmol 5-Hydroxytryptamine (Creatinine sulphate) or 0.15nmol of the 5-HT3 receptor antagonist 3-Tropyl-indole-3-carboxylate (ICS 205-930). The durations of nociceptive response reduction following drug administration are summarised in the table below.

Drug administered	Period for which nociceptive responses were reduced (min.mean ± S.E.M.)
5HT (n=6)	15.9 ± 1.5
ICS 205-930 (n=8)	28.1 ± 7.1
5HT post ICS 205-930 (n=7)	*55.7 ± 17.0

* significantly different 0.05

In this preparation the 5HT3 receptor antagonist failed to reverse the antinociceptive effects of 5HT. Therefore, in contrast to Glaum's results we find no evidence for the involvement of 5HT3 receptors in spinal 5HT antinociception.

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331P INHIBITION BY 5-HT OF IPSILATERAL AND CONTRALATERAL FAST AND SLOW SPINAL REFLEXES IN THE NEONATE RAT

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5-HT pathways project to both dorsal and ventral horns of the spinal cord, but the effects of 5-HT on reflex responses are reported to vary and 5-HT_1, 5-HT_2 and 5-HT_3 receptors are present. More limited actions on reflexes are observed in rat hemisected cord if selective agonists are used (Crick and Wallis, 1991); 5-HT 8-OH-DPAT, TFMPP and methysergide potently depressed the monosynaptic reflex, but a similar action of 5-HT on a polysynaptic reflex was not mimicked by these agents. In 2 day-old rats, contralateral reflexes can be recorded; high threshold afferents elicit slow responses mediated in part by substance P (Akagi et al, 1985). Our experiments tested low concentrations of 5-HT to assess whether any differential action of 5-HT pathways on fast and slow responses (ipsilateral and contralateral) was suggested. The lumbar spinal cord was removed from 1-2 day-old rats anaesthetized with ether and superfused (4-5ml min^{-1}) with Krebs solution at 19-21°C. Glass suction electrodes were used to stimulate a dorsal root and record from ipsilateral and contralateral L_4 Ventral roots. Supramaximal stimuli, 20-30V 0.5ms were applied at 0.07Hz. Three ipsilateral and two contralateral reflexes were identified (Table 1).

		Amplitude (mV)	Latency to peak (ms)	½ decay (ms)
Ipsi- [	Monosynaptic (MSR)	2.5 ± 0.4, n = 10	8.1 ± 0.4, n = 7	-
lateral[	Polysynaptic (PSR)	0.8 ± 0.4, n = 11	22.4 ± 1.7, n = 10	116.5 ± 2.5, n = 11
[	Slow (IPSI SLOW)	0.24 ± 0.06, n = 10	-	7600 ± 1300, n = 9
Contra-[	Fast (CON FAST)	0.24 ± 0.02, n = 11	72 ± 4, n = 9	142 ± 14.7, n = 9
lateral[	Slow (CON SLOW)	0.07 ± 0.01, n = 11	-	8300 ± 3200, n = 8

TABLE 1. IPSILATERAL AND CONTRALATERAL REFLEX COMPONENTS EVOKED BY STIMULATION OF DORSAL ROOT.

All components were blocked by 10μM CNQX; slower components were preferentially depressed by APV (Table 2). All components were depressed by 5-HT or 5-CT. Methysergide, blocked MSR, but only slightly depressed PSR and CON FAST and had no effect on IPSI SLOW and CON SLOW.

	APV 20μM (%↓)	IC_{50} 5-HT μM	5-CT nM	Methysergide nM	Order of potency
MSR	9 ± 2, n = 4	7.6 ± 1.3, n = 7	1.9 ± 0.7, n = 5	25.6 ± 5.5, n = 7	5-CT > METH > 5-HT
PSR	16 ± 3, n = 4	7.8 ± 1.2, n = 8	8.8 ± 2.5, n = 6	-	5-CT > 5-HT >> METH
IPSI SLOW	63 ± 9, n = 4	2.8 ± 0.9, n = 8	3.6 ± 1.4, n = 7	-	5-CT > 5-HT
CON FAST	70 ± 3, n = 4	2.6 ± 0.5, n = 9	3.8 ± 1.3, n = 5	-	5-CT > 5-HT >> METH
CON SLOW	85 ± 4, n = 4	1.2 ± 0.3, n = 7	3.5 ± 1.1, n = 6	-	5-CT > 5-HT

TABLE 2. EFFECT OF APV, 5-HT, 5-CT AND METHYSERGIDE ON IPSILATERAL AND CONTRALATERAL REFLEX COMPONENTS

We conclude that slow reflexes and contralateral reflexes may be modulated by 5-HT and that more than one category of 5-HT receptor may be involved.

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332P EFFECT OF CONCANAVALIN A ON EXCITATORY AMINO ACID-INDUCED DEPOLARIZATION OF DORSAL ROOT C FIBRES

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Concanavalin A has been shown to selectively reduce the desensitization of ∝-amino-5-methyl-4-isoxazole-propionic acid (AMPA)-induced depolarization of hippocampal (Mayer and Vyklicky,1989) and dorsal root ganglion (Huettner, 1990) neurones. The present paper describes this effect at C fibres in isolated dorsal roots from three to five day old rats. Depolarization of C fibres was measured either from depression of the C elevation of the compound action potential or the d.c. shift caused by application of agonists to ther central end of the dorsal root (Agrawal and Evans, 1986). As reported previously C fibres were depolarized for only a few seconds in the presence of L-glutamate at concentrations of 100 μM or higher. Glutamate (125 uM) produced a rightward shift of the kainate concentration-effect plot ($_pA_2$ 5.06 ±0.15 s.e.mean, N=3). The selective agonists quisqualate ($_pA_2$ 5.38 ±0.29) and AMPA ($_pA_2$ 4.53 ±0.14) also produced rightward shifts of the kainate concentration effect plot. Gaddum-Schild slopes for the latter antagonism were significantly less than one, 0.22 and 0.44 respectively. It is likely that, as described for dorsal root ganglion neurones (Huettner 1990), the latter antagonism represents receptor desensitization rather than competitive antagonism. Following treatment of dorsal roots with concanavalin A (10 mg per ml) for 30 to 60 min (in the absence of glucose) AMPA, quisqualate and L-glutamate, but not L-aspartate at concentrations up to 2.5 mM, produced depolarizing responses similar to those produced by kainate. Thus following concanavalin A treatment of four preparations the potency of glutamate was increased approximately twofold and the molar potency ratios of AMPA, glutamate and quisqualate were 0.034 +0.004, 0.074 +0.006, and 0.061 +0.008 respectively relative to kainate=1. At neurones of spinal grey matter AMPA (Krogsgaard-Larsen et al 1980) and quisqualate (Biscoe et al, 1976) have excitatory potencies which are much greater than that of L-glutamate and similar to kainate. Thus lower potencies of AMPA and quisqualate relative to kainate at the present C fibre site suggests that AMPA and quisqualate are displaying weak activity at a receptor which is selectively activated by kainate. The impotency of aspartate suggests that this amino acid may be a selective agonist for a receptor which is not kainate- but which is possibly AMPA-selective.

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333P NEW WILLARDIINE ANALOGUES WITH POTENT STEREOSELECTIVE ACTIONS ON MAMMALIAN SPINAL NEURONES

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Racemates of the naturally occurring uracil-derived amino acid, willardiine, and the corresponding 5-bromo derivative are potent excitants acting at non-N-methyl-D-aspartate (NMDA) type excitatory amino acid (EAA) receptors. We have now prepared the enantiomers of a range of 5-substituted willardiines and investigated their actions at NMDA and non-NMDA type EAA receptors in the spinal cord of the newborn rat.

Compounds were prepared by stereoselective synthesis, and showed high enantiomeric purity (97 to >99%) by chiral chromatography. Recordings of motoneuronal responses were made from ventral roots of hemisected spinal cords of 1-5-day old rats. The substances (0.1-50 µM, administered for 2 min) were tested for (a) their ability to cause depolarization of motoneurones and (b) the susceptibility of such activity to the NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) and the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). The medium was nominally Mg2+-free and contained 0.1 µM tetrodotoxin (TTX).

The (S) forms of all the compounds depolarized motoneurones, with equieffective molar concentrations (relative to (S)-AMPA = 1.0) of: 5-fluoro-,0.33; 5-nitro-,0.65; 5-trifluoromethyl-,0.63; 5-chloro-,1.2; 5-bromo-,1.8; 5-iodo-,2.6; unsubstituted-,7.8; 5-methyl-,32; and 5-methoxycarbonyl-willardiine,47. The corresponding values for other excitants were: domoate,0.7; acromelate A,1.4; quisqualate,2.9 and kainate,4.6. The depolarizations produced by the willardiines were resistant to D-AP5 (20-50 µM) but blocked by CNQX (10-25 µM), indicating that they acted specifically at non-NMDA receptors. (R)-Willardiine, (R)-5-bromowillardiine and (R)-5-iodowillardiine showed only weak agonist activity, consistent with their contents of 0.2%, 1% and 0.2%, respectively, of the corresponding (S)-isomer as determined by chiral HPLC chromatography.

These results indicate the stereospecificity of the action of willardiine analogues at AMPA receptors and show that increasing the acidity of the uracil moiety by the introduction of an electron-withdrawing group in the ring enhances potency. (S)-5-Fluoro-, (S)-5-nitro- and (S)-5-trifluoromethyl-willardiine are all more potent than (S)-AMPA as agonists at the AMPA receptor in this preparation.

334P ACTION OF WILLARDIINE ANALOGUES ON IMMATURE RAT DORSAL ROOTS

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Kainate and (R,S)-5-bromowillardiine cause depolarization of C fibres in dorsal roots of the neonatal rat whereas AMPA and (R,S)-willardiine appear to be relatively ineffective (Agrawal and Evans, 1986). We have investigated a range of new (S)-willardiine analogues for activity at this receptor, including their ability to cause desensitization. Isolated (in vitro) dorsal roots (usually L5) were separated from their point of emergence from the immature rat spinal cord, together with the dorsal root ganglion, as described previously (Agrawal and Evans, 1986) and were placed across a grease seal to record the D.C. potential between peripheral and central ends.

The potencies of the 5-fluoro- (FW), 5-chloro- (CW), 5-bromo- (BW) and 5-iodo- (IW) derivatives of (S)-willardiine (W) as well as W itself, were compared with that of kainate, domoate and (S)-AMPA. 2 ml (1.5 min) applications of agonists (0.05 µM - 2 mM) depolarized dorsal root fibres with relative potencies of (mean ± s.e.mean): domoate, 47.3 ± 2.1 (n=5); IW, 12.8 ± 2.1 (n=13); BW, 3.2 ± 0.4 (n=6); kainate, 1.0; CW, 0.51 ± 0.05 (n=3); FW, 0.035 ± 0.003 (n=4); AMPA, 0.018 ± 0.004 (n=3); W, 0 (n=4). Responses to high doses of IW, BW and CW began to fade during the time of administration which suggests desensitization. Prolonged doses of agonists were used to test for cross-desensitization of kainate and IW, using muscimol as a control, non-excitatory amino acid agonist. The order of potency of desensitization was IW> BW> FW> W which is identical to their rank order for inducing depolarization.

It is concluded that (S)-willardiine analogues desensitize the C fibre kainate receptor with a potency that parallels their depolarizing potency. This rank order is the reverse of that observed in motoneurones (Jane et al., 1991) and in cultured hippocampal cells (Patneau et al., 1991). The ability to desensitize dorsal root fibres is also the reverse order to that for desensitizing AMPA/kainate receptors in cultured hippocampal cells (Patneau et al., 1991). Our data support previous conclusions that kainate receptors in dorsal root fibres of the newborn rat differ not only from AMPA receptors in motoneurones but also from AMPA/kainate receptors in cultured hippocampal cells.

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335P BARBITURATES SELECTIVELY REDUCE DEPOLARISING RESPONSES TO KAINATE RATHER THAN THOSE TO AMPA IN NEONATAL RAT SPINAL CORD *IN VITRO*

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We recently showed that a part of the dose-response curve to kainate in neonatal rat spinal cords was resistant to concentrations of 6-nitro-7-sulphamoylbenzo[f]quinoxalinedione (NBQX) that blocked responses to α-amino-3-hydroxy-4-isoxazolepropionate (AMPA; Zeman & Lodge, 1991). Although this result is consistent with separate receptors for AMPA and kainate distinguished by NBQX and as seen in binding studies (Sheardown et al., 1990), such an interpretation would be strengthened by other selective antagonists. In view of the documented interaction of barbiturates with non-NMDA receptors (Simmonds and Horne, 1988) and of selective reduction of kainate-induced neurotoxicity by phenobarbitone (Frandsen et al., 1990), we have examined the effect of five barbiturates on responses to kainate and AMPA evoked in hemisected spinal cords from neonatal rats (Birch et al., 1988).

All barbiturates tested reduced responses to kainate 5 μM more than those to AMPA 10 μM with methohexitone and phenobarbitone being the most selective and methohexitone the most potent. Methohexitone reduced responses to N-methyl-D-aspartate less than those to AMPA. The rank order of potency against kainate was methohexitone > quinalbarbitone > thiopentone > pentobarbitone > phenobarbitone. For methohexitone, the IC_{50} values versus kainate and AMPA were 31 ± 7 μM (mean ± S.D.) and 172 ± 47 μM respectively. In the presence of 3.16 μM NBQX, 31.6 μM methohexitone almost abolished the NBQX-resistant component of the kainate dose-response curve. In the absence of NBQX, methohexitone 3.16-100 μM shifted the kainate dose-response curve to the right in a dose-dependent but non-parallel manner such that the curve was steepened. Methohexitone 3.16-100μM had little effect on the AMPA dose-response curve whether in the absence or presence of NBQX. This kainate antagonism by barbiturates is unlikely to be explained by their well known GABA potentiating effects, since picrotoxin 50 μM had no significant effect on the shift of the kainate dose-response curve by methohexitone 31.6 μM.

These results indicate pharmacologically distinct receptors by which AMPA and kainate depolarise this neonatal rat spinal cord preparation:- (i) an NBQX-sensitive AMPA receptor which is responsible for most of the action of AMPA and a part of that of kainate and (ii) an NBQX-resistant but methohexitone-sensitive receptor which mediates the remainder of the response to kainate.

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336P CHANGES IN STRIATAL EXTRACELLULAR AMINO ACIDS MEASURED BY INTRACEREBRAL DIALYSIS FOLLOWING INJECTION OF A NEUROTOXIC DOSE OF QUINOLINATE INTO THE RAT STRIATUM

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Injection of the endogenous excitotoxin quinolinate (QUIN) into the rat striatum produces a delayed form of neurodegeneration which can be prevented by administration of an N-methyl-D-aspartate (NMDA) receptor antagonist up to 5 h after QUIN injection (Foster *et al*, 1988). In the present study we have investigated the possibility that changes in extracellular amino acids in the striatum are involved in this phenomenon. Under anaesthesia, male Sprague-Dawley rats were implanted stereotaxically in the right striatum with a dialysis probe (Hutson *et al*, 1985) attached to a guide cannula. The probe was dialysed with artificial CSF (2 μl/min) and 20 min fractions collected for 200 min. QUIN (N=8) or nicotinate (NIC; N=7) (200 nmol in 1 μl over 2 min) were injected into the striatum at a point 1 mm from the dialysis probe using a needle introduced via the guide cannula, and dialysate samples collected for a further 5 h. Amino acid levels were determined by pre-column derivatization with O-phthaldehyde and HPLC with fluorometric detection.

Table 1 Concentrations of amino acids (μM) in striatal dialysates

	Aspartate	Glutamate	Asparagine	Serine	Glutamine	Glycine	Threonine	Arginine
60 min pre-QUIN	0.17±0.01	0.34±0.07	0.16±0.03	2.12±0.25	13.7±1.00	0.78±0.09	1.23±0.18	0.60±0.07
20-40 min post-QUIN	1.04±0.41*	0.65±0.20	0.27±0.05*	4.75±1.26	11.4±1.32	2.65±0.95	1.97±0.40	0.88±0.13
20-40 min post-NIC	0.17±0.07	0.48±0.26	0.12±0.02	1.97±0.41	13.6±1.79	1.22±0.46	1.09±0.11	0.55±0.06

* $P<0.05$, significance of difference from pre-injection and nicotinate controls, paired t-test.

QUIN injection caused an elevation in the levels of aspartate (6x), asparagine (2x), serine (2.5x), glycine (3x) and threonine (2x) which peaked in the fraction 20-40 min after injection, returning to pre-injection values 60-80 min after injection. These changes achieved statistical significance for aspartate and asparagine. Administration of equimolar NIC, which is not neurotoxic (Schwarcz *et al*, 1983), caused no elevation of amino acid levels 20-40 min after injection, but a small increase of some amino acids was observed in the first fraction after injection. Amino acid levels in the dialysate samples remained unchanged during the remaining 5 h after injection of QUIN or NIC. Therefore, extracellular levels of the neuroactive amino acid aspartate are elevated only during the first hour after injection of a neurotoxic dose of QUIN. Since NMDA receptor antagonists give good protection when administered after this time (Foster *et al*, 1988), these acute changes are not directly involved in the neurotoxic mechanism.

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337P SOME INHIBITORS OF Na^+-DEPENDENT QUISQUALATE ACCUMULATION BY RAT HIPPOCAMPAL SLICES

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We have previously shown that accumulation of low μM concentrations of quisqualate (QUIS) by rat hippocampal slices does not have an active component (Harrison & Kilpatrick, 1991a) whereas accumulation of higher QUIS concentrations is sensitive to both temperature reduction and 100μM ouabain (Harrison & Kilpatrick, 1991b). In agreement with recent electrophysiological studies (Frenguelli *et al.*, 1991), this suggests an active component of QUIS transport. Whilst excitatory amino acid (EAA) transport appears to occur via Na^+-dependent sites and a Ca^{2+}/Cl^--dependent carrier, we report here some characteristics of Na^+-dependent QUIS accumulation.

QUIS content of slice homogenates was assayed following incubation of 400μm rat hippocampal slices with QUIS at 25°C. Mean values of QUIS content obtained on equimolar replacement of extracellular Na^+ by choline are subtracted from mean control values to give an estimate of the Na^+-dependent component. The concentration-accumulation relationship of this component (CONTROL, ○) is shown in Figure 1. An iterative fitting of the data to a logistic expression gave an estimated V_{max} of 1.81 nmol/slice/4 min and a K_m of 990 μM. The known inhibitors of Na^+-dependent EAA transport, *threo*-ß-hydroxy-L-aspartate (TßHLA; Fig. 1, ♦) and L-*trans*-pyrrolidine-2,4-dicarboxylate (L-*trans*-PDC; Fig. 1, □), each at 1 mM, completely inhibited Na^+-dependent accumulation, until the QUIS concentration exceeded 2 mM. L-Homocysteate (L-HCA) is known to be a substrate for the low- but not the high-affinity Na^+-dependent EAA transporter (Cox *et al.*, 1977). The effect of L-HCA (Fig. 1, ▲), on Na^+-dependent QUIS uptake together with the high K_m for QUIS transport, infer that the site responsible for Na^+-dependent QUIS accumulation is of the low-affinity type. A possible effect of L-HCA on the Ca^{2+}/Cl^--dependent site should not be excluded, as may be evinced by its inhibition of QUIS uptake beyond that due to the Na^+-dependent component.

We conclude that Na^+-dependent QUIS accumulation by these slices is 1) saturable, 2) of low affinity and 3) resembles a similar site described for the endogenous EAA, L-glutamate (Cox *et al.*, 1977).

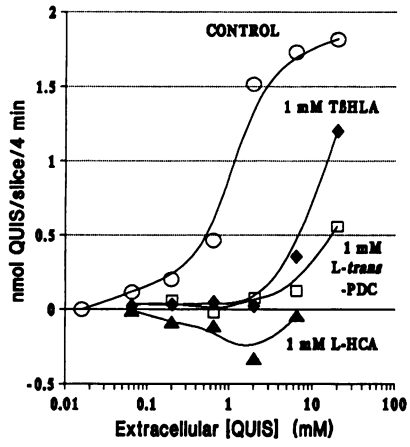

Figure 1. Influence of EAA transport inhibitors on Na^+-dependent accumulation of QUIS into rat hippocampal slices. Each point is the mean of 4-14 experiments.

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338P MODULATION OF [^{3}H]CPP BINDING BY NMDA RECEPTOR COMPLEX LIGANDS

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The N-methyl-D-aspartate (NMDA) excitatory amino acid receptor complex is composed of multiple functional components. The competitive antagonist [^{3}H]3-((+)-2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP) is highly suitable for labelling the NMDA receptor in both membrane binding and autoradiographic studies. We previously demonstrated that using a centrifugation assay, [^{3}H]CPP interacts with two binding sites on rat brain synaptic membranes (Porter et al., 1990). These two binding components may represent NMDA receptor subtypes as proposed by Cotman et al. (1989). There is considerable evidence for reciprocal modulation between the various sites on the NMDA receptor complex. The best studied interaction to date, is that between the NMDA/glycine/PCP sites (see Cordi et al., 1990). Following an observation by Pullan et al. (1990) of the ability of 1-hydroxy-3-amino-pyrrolid-2-one (HA-966) to increase [^{3}H]CPP binding, we have investigated the ability of glycine site agonists and antagonists and polyamines for their ability to influence the binding of [^{3}H]CPP.

Extensively-washed whole rat brain synaptic membranes (minus cerebellum) were incubated for 30 min at 20°C in Tris-acetate buffer with [^{3}H]CPP (20 nM). Non-specific binding was defined with 1mM unlabelled AP5 or AP7. Glycine itself was found to produce a very weak enhancement of specific [^{3}H]CPP binding. Both enantiomers of the glycine site antagonists HA-966 (R-HA-966, EC_{50} = 5.0 ± 0.4µM; S-HA-966, EC_{50} = 164 ± 26µM) and 1-aminocyclobutanecarboxylic acid (ACBC) (EC_{50} = 22 ± 3µM) produced a substantial enhancement in binding by increasing the affinity of [^{3}H]CPP for its binding sites. The effects of HA-966 and ACBC could be competitively antagonised by glycine. This enhancement of [^{3}H]CPP binding is not however a general property of glycine site antagonists. A similar mechanism operates for the enhancement of [^{3}H]CPP binding by the polyamine agonists, spermine and spermidine (EC_{50}s= 10µM and 39µM respectively). The enhancement was not antagonised by the conventional polyamine antagonists cadaverine and DET (diethylenetriamine), suggesting that a different site is involved from that which modulates [^{3}H]MK-801 binding. The enhancement by spermine and the glycine site antagonists was additive, indicative of their acting at discrete sites. Although functional data in support of a physiological role of this NMDA receptor modulation are lacking, it may present potential therapeutic targets in neuropathologies involving NMDA receptors.
R.H.P. Porter is a Research into Ageing Research Student.

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339P THE STIMULATION OF DOPAMINE METABOLISM BY DIZOCILPINE (MK-801) IS BLOCKED BY R(+)-HA-966

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The glycine/NMDA receptor antagonist, R(+)-HA-966 (R(+)-3-amino-1-hydroxypyrrolid-2-one), diminishes the stimulant actions of the non-competitive NMDA receptor antagonist, phencyclidine (PCP) on mesolimbic dopamine (DA) metabolism in the rat (Hutson et al., 1991). Unlike PCP, dizocilpine (MK-801) has high selectivity for the NMDA receptor. In the present study, we show that (+)-HA-966 also antagonises the stimulation of mesolimbic dopamine metabolism and motor activity induced by dizocilpine.

Male Sprague-Dawley rats (280 - 310 g) were pretreated with either (+)-HA-966 (30 mg/kg, i.p.) or vehicle (0.9% NaCl). After 20 min, either dizocilpine (0.5 mg/kg, i.p.) or saline was administered. One hour later the rats were humanely euthanased and the striatum, nucleus accumbens (N.AC), medial pre-frontal cortex (PFX) and amygdala were dissected and analysed for DA, DOPAC and HVA using HPLC with ECD.

Table 1. ([DOPAC]+[HVA])/[DA]

	STRIATUM	N.AC	PFX	AMYGDALA
Saline/saline	0.24 ± 0.007	0.29 ± 0.008*	0.80 ± 0.07*	0.26 ± 0.02*
Saline/dizocilpine	0.25 ± 0.007	0.46 ± 0.02	1.78 ± 0.14	0.49 ± 0.03
(+)-HA-966/saline	0.22 ± 0.007	0.27 ± 0.009*	0.83 ± 0.12*	0.23 ± 0.01*
(+)-HA-966/dizocilpine	0.23 ± 0.008	0.36 ± 0.01*	1.03 ± 0.12*	0.29 ± 0.02*

Values are means ± s.e means of 6 - 12 animals per group. * $p<0.05$ c.f. saline/dizocilpine group (ANOVA)

Dizocilpine significantly increased dopamine turnover, without affecting dopamine concentration, in the N.AC, amygdala and PFX, but not in the striatum. (+)-HA-966 alone did not alter dopamine turnover or concentration in any brain region, but significantly attenuated the response to dizocilpine in all areas (Table 1). Dizocilpine (0.4 mg/kg, s.c.) also stimulated motor activity in 20 - 30 g male BKTO mice in automated photocell activity cages (mean photocell beam breaks per 2 h ± s.e. mean of 8 animals per group, commencing immediately after injection = 10 ± 2 and 679 ± 146 in saline and dizocilpine-treated mice respectively). (+)-HA-966 (30 mg/kg, s.c.) given 30 min before did not alter motor activity scores (13 ± 5), but significantly reduced the stimulation induced by dizocilpine (213 ± 67, $p < 0.05$).

Thus, the results indicate that the activation of mesolimbic DA systems by PCP involves blockade of the NMDA receptor and suggest that this activation can be differentially influenced by drugs acting at the glycine modulatory site and the ion channel of the NMDA receptor complex.

Hutson, P.H., Thorn, L., Bristow, L. and Tricklebank, M.D. (1991) Brit. J. Pharmacol., 102, 67P.

340P FUNCTIONAL DIVERSITY OF NON-NMDA RECEPTORS EXPRESSED FROM AVIAN AND MAMMALIAN BRAIN MRNA-INJECTED *XXXENOPUS LAEVIS* OOCYTES

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The properties of non-NMDA receptors were studied in *Xenopus laevis* oocytes microinjected with mRNA extracted from 3 month calf (cortex) brain, 1 day chick whole brain, or 3-6 week rat whole brain. A two-electrode voltage clamp technique was used to investigate functional properties of the expressed receptors, including: **(i)** the agonist current-voltage (I-V) characteristics, **(ii)** concentration-response relationships and **(iii)** the relative level of receptor expression as a function of the agonist-induced response amplitude.

I-V relationships (-160mV to +60mV) produced by quisqualic acid (QA; 6μM, n=8) or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA; 100μM, n=7) in all oocytes, were characterised by outward rectification, particularly at positive membrane potentials. In contrast, I-V relationships to kainic acid (KA) were dependent on the species of mRNA. Using calf (n=9) or chick (n=7) brain mRNA, the response to KA (80μM) exhibited inward rectification at positive membrane potentials (>+20mV), whereas outward rectification occurred with rat brain mRNA-injected oocytes (n=6). Log dose-response (D/R) curves for KA (5μM-640μM) were similar for calf, chick and rat mRNA expressed receptors with estimated K_d values of 60.2 ± 4μM (mean ± sem; n=34), 62.2 ± 10μM (n=6) and 75 ± 9μM (n=5) respectively. With each mRNA species, the KA-evoked current (80μM) was large and non-desensitizing (e.g. calf; 51 ± 5nA, n=27). The responses evoked by AMPA (2.5μM-200μM, n=4) were also quite similar in all oocytes (e.g chick; EC_{50} = 4μM); however, AMPA (e.g. 100μM; 3.2 ± 0.6nA, n=11; calf) and QA responses (e.g. 6μM; 4 ± 0.7nA, n=13; calf) were much smaller, compared to KA, and exhibited desensitization.

KA-induced currents (80μM) were reduced by co-application of either 100μM AMPA (e.g. calf, 55 ± 5% inhibition, n=5) or 6μM QA (e.g. calf, 70 ± 3%, n=18) in all mRNA injected oocytes. Using calf or chick brain mRNA, QA (6μM; n=8) or AMPA (100μM; n=7) antagonised the KA D/R curve in a non-competitive manner. The antagonism also occurred in the occasional absence of any measurable response to QA (n=3). With rat brain mRNA, AMPA (n=3) antagonised the KA D/R curve in a competitive manner without any modification to the maximal-response. The response-amplitudes to QA (6μM), AMPA (100μM) and KA (80μM) were compared in each oocyte. Using rat brain mRNA (n=9), a direct correlation between the amplitudes of KA and AMPA responses existed, consistent with both agonists *sharing* the same receptor-ionophore. In contrast, using calf (n=26) or chick (n=13) brain mRNA, there was no clear correlation between the responses to QA or AMPA with KA.

We suggest that the expressed non-NMDA receptors were equi-sensitive to KA but exhibited functionally diverse properties, which may indicate an underlying receptor heterogeneity.

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341P EPILEPTIFORM DISCHARGE MEDIATED EXCLUSIVELY BY NON-NMDA RECEPTORS IS DEPRESSED BY 5-HT IN RAT ENTORHINAL CORTEX *IN VITRO*

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Previous studies from this laboratory have demonstrated that 5-hydroxytryptamine (5-HT) is predominantly a depressant neuromodulator of excitatory amino acid (EAA) responses in rat entorhinal cortex (EC; Sizer & Roberts, 1990a). Epileptiform discharge in the EC induced by perfusion of the GABA antagonist bicuculline has been shown to be mediated by EAAs (Jones & Lambert, 1989), and subsequently 5-HT potently depresses epileptiform discharge in this region (Sizer & Roberts, 1990b). At low concentrations (<10uM) 5-HT can exert its depressant effect in two different ways (Sizer & Roberts, 1991). The most common effect is to reduce the length of individual bursts, but 5-HT can also potently reduce the frequency of spontaneous bursting.

The predominant action of 5-HT when inhibiting burst length is to abolish the after-discharges associated with the burst and these after-discharges have been shown to be mediated by NMDA receptors (Jones, 1988). Consequently, it was thought that 5-HT might be exerting a specific depressant action on the NMDA receptor.

Intracellular recordings were made in layers II/III of the entorhinal cortex and epileptiform activity was induced by adding 5uM bicuculline to the perfusate. The discharge generally became stable within thirty minutes. 5-HT (5 minutes perfusion at 10uM) was first tested on control epileptiform discharge to examine its effect. Once the 5-HT had washed out, the NMDA antagonist D-APV was added to the perfusate at a concentration of 50uM (a concentration that has previously been suggested to block all physiologically activated NMDA receptors, Collingridge et al., 1988). This remaining discharge was completely abolished by the non-NMDA antagonist CNQX (10uM). 5-HT was re-tested on this CNQX-sensitive epileptiform discharge. In all cases 5-HT was still able to depress further the remaining activity.

In summary, therefore, it seems that 5-HT is able to depress synaptically-activated non-NMDA responses in a moderately potent manner. The 5-HT receptor mediating this effect is currently under investigation.

A.R.S. is a Glaxo Research Scholar

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collagen, thrombin or ADP

342P INSULIN-LIKE GROWTH FACTOR-1 POTENTIATES PLATELET AGGREGATION INDUCED BY COLLAGEN, THROMBIN OR ADP

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Thrombin induces the release of IGF-1 from human platelet alpha granules. Receptors for IGF-1 and also insulin have been identified on platelets (Hartmann et al 1989, Karey et al 1989) and although their physiological function is unknown, insulin has been shown to reduce platelet sensitivity to a number of aggregants. Here we report that IGF-1 enhances platelet aggregation induced by thrombin, collagen or ADP.

Washed platelet suspension (WPS) or platelet rich plasma (PRP) obtained from fasted male and female volunteers (aged 21-40 years) and prepared according to the method of Radomski and Moncada (1983) was preincubated at room temperature with IGF-1. 500μl aliquots were subsequently incubated for 1 min at 37°C in a Payton dual channel aggregometer with continuous stirring at 1000rpm, prior to challenge with platelet agonist. The degree of potentiation was measured as increases in peak response to doses of agonist producing 20% or 50% platelet aggregation. IGF-1 caused an almost immediate and dose dependent potentiation of both collagen (0.2-0.5μg/mL) and thrombin (10-30mU/mL) induced aggregation of WPS. The threshold dose for the effect was 30nM. Maximal potentiation (ranging from 50-100% above control) was observed at IGF-1 concentrations between 0.1 and 0.3μM although the degree of potentiation exhibited marked inter-individual variation. Potentiation was seen after preincubation with IGF-1 from 30 s up to 50 min prior to agonist challenge. Potentiation of platelet aggregation by IGF-1 was also observed in PRP after challenge with ADP (0.5-2μg/mL), though the responses were smaller (20-40% potentiation of peak response) than those observed with WPS.

IGF-1 preincubation also significantly potentiated increases in intra-platelet free calcium induced by thrombin (24$\pm$12%) and collagen (57$\pm$22%) in WPS, as measured by fluorimetry after loading of platelets with the fluorescent calcium label, fura 2. IGF-1 did not affect basal calcium levels. There was no effect of insulin preincubation on platelet aggregation or on agonist induced changes in intra-platelet calcium at doses up to 1μM.

IGF-1 expression is increased in response to tissue injury. Our studies suggest that IGF-1 may play a role in modulating platelet reactivity, possibly via a mechanism which modifies agonist induced changes in intra-platelet free calcium. This may be of importance in disease states where platelets are involved e.g. thrombosis.

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343P CULTURED ENDOTHELIAL CELLS CONVERT N^G-HYDROXY-L-ARGININE TO NITRIC OXIDE

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Endothelium-derived relaxing factor or nitric oxide (·NO), released from the vascular endothelium by a variety of stimuli, is a potent vasodilator and antithrombotic agent. The formation of ·NO from L-arginine (L-Arg) is catalyzed by a NADPH-dependent dioxygenase, collectively referred to as ·NO-synthase, which can exist in at least two distinct forms. One isoenzyme, a constitutive, agonist-triggered and calcium/calmodulin-dependent ·NO-synthase, is mainly present in neuronal cells and endothelial cells, whereas the other isoenzyme, a calcium-independent inducible ·NO-synthase, is found predominantly in macrophages and smooth muscle cells. Recently, N^G-hydroxy-L-arginine (L-HOArg) was identified as an intermediate in the biosynthesis of ·NO from L-Arg by activated macrophages (Stuehr *et al.*, 1991). As L-HOArg may not only be a useful tool in studying the reaction mechanism of ·NO-synthase but also its cellular regulation, we have investigated whether L-HOArg is also an intermediate in the biosynthesis of ·NO by endothelial cells. ·NO release from bovine aortic EC on microcarrier beads was determined by using the superfusion bioassay cascade technique. When infused through the column of EC (t.c.), both L-HOArg and D-HOArg (1-10 μM), in the presence (10-30 units/ml) or absence of superoxide dismutase, substantially (up to 10-fold) potentiated relaxations of the detector tissues to ·NO released from the column of EC following stimulation with ADP or bradykinin. This effect was inhibited by N^G-nitro-L-arginine (L-NO_2Arg) methyl ester (10 μM) or N^G-monomethyl-L-arginine (L-MeArg; 30 μM). Both L-HOArg and D-HOArg, irrespective of the presence of EC, also potentiated relaxations of the detector tissues induced by authentic ·NO, but not glyceryl trinitrate, suggesting that they did not affect the release of ·NO but prolonged its half-life. Indeed, L-HOArg reacted directly with ·NO to form a potent and more stable vasodilator substance which, according to chemiluminescence analysis, readily released ·NO. Consequently, infusions of L-HOArg (3 μM) t.c. were made in the presence of D-HOArg (10 μM) to distinguish between this non-specific chemical reaction and a genuine effect on ·NO biosynthesis. Under these conditions, L-HOArg no longer had any effect on relaxations induced by authentic ·NO, but significantly (2-fold) potentiated the stimulated release of ·NO from the column of EC. The conclusion that L-HOArg, therefore, may be an intermediate in the biosynthesis of ·NO by EC was strongly supported by the conversion of L-HOArg, but not D-HOArg, to ·NO by ·NO synthase preparations from these cells. The activity of the constitutive, predominantly membrane-bound enzyme was determined by monitoring the ·NO-mediated increase in intracellular cyclic GMP in LLC-PK_1 pig kidney epithelial cells. Both L-HOArg and L-Arg (100 μM), but not D-HOArg or D-Arg, caused a significant (2 to 3-fold) increase in cyclic GMP which was completely inhibited by equimolar concentrations of L-NO_2Arg. Interestingly, t.c. infusions of L-HOArg (1-10 μM), but not D-HOArg, also elicited a stable relaxation of the detector tissues which was blocked by oxyhaemoglobin (0.3-3 μM), but not by L-MeArg or L-NO_2Arg methyl ester (10-100 μM). (***Supported by a grant from Glaxo Group Research Ltd.***)

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344P EFFECTS OF EDRF SYNTHESIS INHIBITORS ON RAT PAW OEDEMA

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The EDRF synthesis inhibitor, N^G-monomethyl-l-arginine (L-NMMA; Rees et al., 1989) inhibits polycation-induced oedema but this inhibition is reversed by the prostacyclin analogue Iloprost, indicating an effect on flow rather than decrease in vascular permeability (Antunes et al., 1990). Here we compared the effects of L-NMMA and nitro-arginine-methyl-ester (L-NAME; Moore et al., 1990) on bradykinin (Bk), serotonin (5-HT) and compound 48/80-induced rat paw oedema. The effect of sodium nitroprusside (SNP) on **in vitro** histamine-releasing activity of mixed peritoneal cells was also investigated.

Oedema was induced by a single sub-plantar injection of Bk (50 nmol/paw), 5-HT (17 nmol/paw) or 48/80 (3-10 μg/paw) into the left hind-paw of rats under light ether anaesthesia. The paw volume was measured immediately before the injection and at set time intervals afterward using a hydroplethysmometer (model 7150, Ugo Basile, Italy). The final volume injected into the paw was 0.1 ml. Results (mean ± S.E.M.) were expressed as percentage change of paw volume. Depletion of histamine and 5-HT stores was undertaken by repeated injections of 48/80 as previously described (Di Rosa et al., 1971). Mixed peritoneal cells were recovered from male rats by lavage with Krebs' solution. Aliquots of cells were equilibrated at 37°C for 10 min and then incubated with SNP and/or 48/80. Histamine release was allowed to proceed for a further 10 min and the amounts (expressed as a percentage of the total cellular content of the amine) determined by fluorometric method (Shore et al., 1959).

L-NMMA (7.5-15 μmol/paw, n=7) alone did not induce paw oedema but significantly ($p<0.05$) reduced oedema induced by Bk (63.5 ± 2.4%, n=10), 48/80 (55.7 ± 8.7%, n=5) and 5-HT (26.1 ± 4.2%, n=5). L-NAME (15 μmol/paw) potentiated Bk (75.0 ± 8.4%, n=10), 5-HT (34.0 ± 6.4%, n=10) and 48/80 (39.4 ± 13.1%, n=10)-induced oedema. This potentiation was abolished either by pre-treating the animals with a mixture of mepyramine (6 mg kg^{-1}) and methysergide (6 mg kg^{-1})or by depleting their stores of histamine and 5-HT. Similarly, co-injection of SNP (40 nmol/site) also reduced (85.5 ± 18.3 %, n=5) the potentiation caused by L-NAME on 48/80-induced oedema. Incubation of SNP (10 μM) with mixed peritoneal cells inhibited basal (from 10.2 ± 1.1% to 2.8 ± 2.0%, n=5; $p<0.05$) but not 48/80 (0.25-1 μg/ml, n=5)-induced histamine-releasing activity.

Our results clearly show that L-NMMA and L-NAME have a different profile on rat paw oedema. In contrast to L-NMMA, L-NAME potentiated Bk, 48/80 and 5-HT -induced oedema. This potentiation could be due to histamine/5-HT released in response to mast cell activation, since mepyramine/methysergide treatment as well as depletion of histamine/5HT stores abolished this effect. It is interesting that SNP inhibited both the potentiation induced by L-NAME and the basal release of histamine from mixed peritoneal cells. Since SNP is a nitric-oxide (NO) mimetic and peritoneal mast cells synthesize NO (Salvemini et al., 1990), These results indicate that the NO-cGMP system may be involved in the modulation of mast cell degranulation.

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345P TOXIC SHOCK SYNDROME TOXIN-1 INDUCES NO-SYNTHASE IN A MACROPHAGE-LIKE CELL LINE

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Staphylococcal toxic shock syndrome toxin-1 (TSST-1) is an exotoxin from a family of staphylococcal enterotoxins (SEs) (Schlievert, P.M at al, 1981) which are the most common cause of food poisoning. TSST-1 has been implicated in the pathogenesis of toxic shock syndrome, a multisystem disease characterized by hypotension, high fever, erythroderma, desquamation of skin, and dysfunction of three or more organ systems (Todd, J. et al, 1978). TSST-1 as well as SEs are potent activators of T lymphocytes in the presence of cells bearing MHC class II antigens. SEs and TSST-1 also induce immunosupression and prevent tumour growth *in vivo*.

We investigated the hypothesis that TSST-1 induces NO-synthase leading to an increased generation of NO which then contributes to the hypotension observed in patients with toxic shock syndrome, and perhaps to other immunoregulatory effects of TSST-1. Here, we demonstrate the induction by TSST-1 of NO-synthase activity in the mouse macrophage-like cell line J774.

J744 cells were cultured in 6-well dishes. After 24 h incubation with or without TSST-1, cells were harvested,washed, and assayed for NO production using the cGMP response in LLC-PK_1 pig kidney epithelial cells. Accumulation of nitrite in supernatants was measured by the Griess reaction (Griess, P., 1879). J774 cells treated with TSST-1 (10-100 nM) caused concentration-dependent elevations of cGMP in the LLC-PK_1 cells ($p<0.0001$) as well as the accumulation of nitrite in the medium ($p<0.001$). Elevations of cGMP in the LLC-PK_1 cells were completely abolished by oxyhaemoglobin (5 mM, $p<0.001$)) or methylene blue (10 mM, $p<0.001$) and potentiated by superoxide dismutase (100 U/ml, 85 $\pm$ 5 %inhibition, $p<0.01$). NO production by J744 cells was arginine-dependent for it was inhibited by preincubation (20 min) of J744 cells with MeArg (300 mM, 88 $\pm$ 1 %inhibition, $p<0.001$), arginine-free medium containing D-arginine (0.4 mM, 80 $\pm$ 3 %inhibition, $p<0.001$). The induction of NO-synthase activity was also detected in cytosol prepared from J774 cells incubated with TSST-1.
These effects were not due to contamination of the TSST-1 with endotoxin which was less than 0.3 mIU/mg TSST-1 as assayed by the limulus test. Furthermore, induction of NO-synthase in J744 cells by TSST-1 was prevented by a specific anti-TSST-1 antiserum.

Thus, TSST-1 and perhaps other exotoxins produced by Gram-positive bacteria induce NO-synthase and the increased NO formation may contribute to toxic shock syndrome and possibly to changes in the immune responses which accompany infection.(This work was supported by a grant from Glaxo Group Research Ltd.)

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346P INHIBITION OF CYTOKINE ACTION BY LIPOCORTIN-1 (ANNEXIN-1): IMPLICATIONS FOR MECHANISMS OF ACTION

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We have provided evidence to suggest that lipocortin-1 is an endogenous inhibitor of the pyrogenic and thermogenic actions of interleukin-1ß (IL-1ß) in the rat (Carey et al., 1990). Anti-inflammatory and antipyretic actions of lipocortin-1 have been ascribed to inhibition of eicosanoid synthesis. However, the effects of IL-1ß are also dependent on corticotrophin releasing factor (CRF). The objective of this study was to compare the effects of lipocortin-1 on responses to IL-1ß and IL-6, which are related to CRF release, with those of IL-1α and tumour necrosis factor α (TNFα) which are independent of CRF, and IL-8 which is dependent on CRF, but not on cyclooxygenase products (Rothwell, 1991).

Fever, (rise in colonic temperature) and thermogenesis (VO_2, indirect calorimetry) were measured in conscious rats in response to central (icv) injections (via indwelling guide cannulae) of maximal doses of recombinant cytokines, with either vehicle (0.9% saline) or a recombinant fragment (N-terminal 1-188aa) of human lipocortin-1 (1.2 μg,icv). All cytokines elicited significant increases in VO_2 (13-20 %) and colonic temperature (0.7-1.6°C), which were maximal after 90-120 min. Vehicle or lipocortin-1 caused small increases in Vo_2 (<5%) and temperature (up to 0.4°C). Pretreatment with lipocortin-1 markedly ($P<0.05$) attenuated the responses to IL-1ß (5 ng; inhibition: VO_2 70%, temp. -1.1°C), IL-6 (20 ng; VO_2 60%; temp -1.0°C) or IL-8 (20 ng VO_2 23%, temp -0.4°C) However lipocortin caused only slight (non-significant) inhibition of responses to TNFα (1μg; inhibition VO_2 12%, temp 0.1°C) or IL-1α (50ng, inhibition VO_2 0%, temp. +0.2°C). Thus, lipocortin-1 modified responses only to those cytokines which are dependent on CRF. Actions of all of the cytokines tested with the exception of IL-8, can be prevented by cyclooxygenase inhibitors. Therefore it is unlikely that lipocortin-1 acts by reducing prostaglandin synthesis. In separate rats, injection of CRF_{41} (icv, 5 μg) stimulated VO_2 (27%), and this effect was markedly attenuated (60%) by pretreatment (icv) with lipocortin-1. Lipocortin-1 therefore appears to inhibit directly the thermogenic responses to CRF; this may explain its selective modification of cytokine action.

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347P CHRONIC ETHANOL STIMULATES LIPOGENESIS IN BROWN ADIPOSE TISSUE OF NORMAL AND OBESE DIABETIC CBA/Ca MICE

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Brown adipose tissue (BAT) has a greater capacity for lipogenesis than white adipose tissue (WAT) and its response to insulin may be markedly higher. In genetically obese (ob/ob) mice, the level of BAT lipogenesis is reduced (Mercer & Trayhurn, 1983). Since chronic ethanol treatment has been reported to reverse the diabetic condition in CBA/Ca mice (Connelly & Taberner, 1986; 1989), the aim of this study was to determine whether lipogenesis, which is reduced in the obese condition, is affected by chronic ethanol treatment.

Lipogenesis rates were estimated *in vivo* by measuring the incorporation of ^3H into fatty acids extracted from adipose tissue following i.p. injection of ^3H_2O, using the methods described by Mercer & Trayhurn(1983). Mitochrondrial activity of BAT was monitored by measuring the specific activity of cytochrome oxidase. Chronic ethanol was administered in the drinking water as a 20% ethanol solution for 4 weeks, as the sole fluid available to the mice.

TABLE 1 Lipogenesis rates (means ± SEM, n) μg atoms H incorporated/hr/g fat free tissue weight

	BAT		WAT	
	untreated	20% ethanol	untreated	20% ethanol
Control lean	113.9±9.9(11)	130.1±7.9(12)	23.0±3.7(12)	22.8±4.4(12)
Control obese	63.7±10.(13)	102.2±8.1(11)	33.6±7.1(12)	20.1±4.9(12)
Insulin-stimulated lean	162.0±19.3(20)	373.2±57.7(10)	22.6±4.2(16)	49.6±11.6(11)
Insulin-stimulated obese	145.6±20.1(18)	199.3±37.9(11)	43.6±11.6(16)	60.8±13.7(11)

Obese mice showed significantly lower lipogenesis in BAT but not WAT compared to lean controls ($p<0.002$, t-test). BAT lipogenesis in ethanol-treated obese mice was significantly increased ($p<0.01$) above control obese but there was no change in WAT. Insulin (1 IU/kg i.p.) in ethanol-treated obese mice further increased lipogenic rates in BAT significantly above the basal (control) rate ($p<0.02$). The specific activity of cytochrome oxidase was not significantly different from control lean mice 1710±168 (8) μmol cyt c/min/mg protein in any of the other groups. This suggests that the lower lipogenesis in obese mice is not associated with reduced mitochondrial oxidative activity and that ethanol does not stimulate lipogenesis by increasing mitochondrial oxidative activity.

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348P CONTRASTING EFFECTS OF ELEVATED EXTRACELLULAR CALCIUM AND A CALCIUM IONOPHORE ON ISOLATED NEONATAL RAT AND QUAIL MEDULLARY BONE OSTEOCLASTS

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In birds, medullary bone is a gonadal steroid dependent form of endosteal bone found within marrow cavities of long bones and is a specific adaptation to the requirements of calcium metabolism during egglay (Dacke 1979). We have studied acute effects of elevated extracellular calcium ($[Ca^{++}]_e$) and a calcium ionophore, ionomycin on the morphology and intracellular calcium ($[Ca^{++}]_i$) levels of osteoclasts freshly isolated from medullary bone of egglaying Japanese quail and neonatal rat femura. Medullary bone of 3 to 6 month old quail hens (15h post-ovulation and following an overnight fast) or 1 to 2 day old rat femora were mechanically disaggregated into Medium 199 and the resulting suspension gently pipetted onto tissue culture wells . The cells were allowed to settle and attach for between 20 and 60 min at 37^0 C. After a wash , followed by a 20min re-incubation to allow spreading , the cells were visualized by video-enhanced phase-contrast microscopy. Time-lapse video recording revealed the presence of large multinucleate osteoclasts with actively ruffling edges. For the measurement of cell motility and spreading we have used a computer programme that depends on digitisation of cell images at timed-intervals , redigitisation of the cell margin, and analysis of shape change. $[Ca^{++}]_i$ was measured in single osteoclasts by a dual-emission microspectrofluorimetric method, using indo-1. The exposure of isolated rat osteoclasts to elevated $[Ca^{++}]_e$ (20mM) led to a marked reduction of osteoclast spread area at 22min to 54.6% ±4.6(s.e.mean,n=5) of the original cell size. This was followed by spontaneous recovery even in the continued presence of elevated $[Ca^{++}]_e$. The half time of the response was 10min. In contrast quail medullary bone osteoclasts showed no reduction of cell spread area up to 30 min post-exposure $[Ca^{++}]_e$ (5-20mM). There was no evidence of cytotoxicity. Measurements of $[Ca^{++}]_i$ revealed that whilst elevated $[Ca^{++}]_e$ caused a concentration dependent elevation of $[Ca^{++}]_i$ in rat osteoclasts, no such response was obtained for the quail cells. However, exposure of both rat and quail medullary bone osteoclasts to ionomycin (10-100μM) caused a sustained elevation in $[Ca^{++}]_i$ that was dose dependent. Exposure of rat and quail osteoclasts to 1μM and 7μM ionomycin respectively caused a significant reduction in cell spread area to 61.0% ±5.0(s.e.mean,n=5) and 84.1% ±5.7 (s.e.mean,n=4) of the original cell size. The half time of these responses were 5min for rat and 10min for quail osteoclasts. These findings suggest that quail medullary bone osteoclasts, like neonatal rat cortical bone osteoclasts, possess contractile processes that can be triggered into operation by an elevation in $[Ca++]_i$. However there are differences in the responsivness of rat and quail osteoclasts to elevation of $[Ca^{++}]_e$. This indicates that under these conditions quail medullary bone osteoclasts either do not possess the Ca^{++} sensing mechanism that is believed to be present in rat cortical osteoclasts (Zaidi 1990) or that it is heavily down regulated in the former species.

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349P INVESTIGATION OF THE 5-HT RECEPTOR TYPES CONTROLLING THE SHORT-CIRCUIT CURRENT RESPONSE IN GUINEA-PIG ILEAL MUCOSA

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In guinea-pig ileal mucosa 5-hydroxytryptamine (5-HT) stimulates a biphasic increase in short-circuit current, an index of electrogenic anion secretion. The initial "spike" response is transient, sensitive to tetrodotoxin (TTX) and blocked by 5-HT_3 receptor antagonists (Cooke and Carey, 1985; Hendricks et al., 1989). The second phase of the response to 5-HT is well maintained and unaffected by TTX. We have investigated the 5-HT receptor(s) mediating this latter response.

The mucosa of guinea-pig ileum was dissected from the overlying muscle layers and mounted between the two halves of an Ussing chamber. The tissues were bathed bilaterally with Krebs-Henseleit solution at 37°C and gassed with 95% O_2/5% CO_2. The potential across the mucosa was clamped at zero and the short-circuit current (SCC) continuously recorded. Tissues were routinely treated with TTX (0.3μM) to inhibit the "spike" response to 5-HT and with 3-isobutyl-1-methylxanthine (10μM) to reduce the concentration of 5-HT required to stimulate the maintained response. All drugs were added bilaterally.

In paired preparations of ileal mucosa 5-HT (0.03-30μM) stimulated reproducible concentration-related increases in SCC with an EC_{50} value of 0.9 (0.4-1.9)μM (95% confidence limits) and a maximum increase in SCC above basal of 101.3±5.5μAcm^{-2}. Several indole and benzamide compounds were tested as agonists and their activities compared with 5-HT (see table).

Compound	*EC_{50} (μM)	% 5-HT maximum	Compound	*EC_{50} (μM)	% 5-HT maximum
5-HT	0.9 (0.4-1.9)	100	Renzapride	0.5 (0.4-0.8)	57±11
5-MeOT	2.6 (2.1-3.1)	116±17	R,S-Zacopride	1.5 (0.9-2.3)	48±3
α-methyl-5-HT	9.1 (7.4-11.1)	79±2	Cisapride	1.9 (0.6-6.0)	19±8
5-CT	10.6 (7.0-16.2)	110±9	Metoclopramide	15.4 (12.7-18.7)	17±3
			2-methyl-5-HT	94.7 (43.2-207.9)	53±4

*EC_{50} values calculated using own maximum for each agonist. 5-MeOT=5-methoxytryptamine; 5-CT=5-carboxamidotryptamine

The rank order of potency of the indole agonists and the partial agonist activity of the benzamides is consistent with the presence of the putative 5-HT_4 receptor (Craig and Clarke, 1990).

A number of antagonists had no effect against the 5-HT-induced SCC response [ketanserin (1μM), methysergide (10μM), methiothepin (0.3μM), ondansetron (10μM), ICS205-930 (10μM)]. However, ICS205-930 (10μM) was tested against some of the more selective agonists described above, and parallel rightward shifts were obtained from which the following apparent pK_B values were calculated: R,S-zacopride 6.3±0.1, 5-MeOT 5.8±0.2, 5-CT 6.0±0.1, α-methyl-5-HT 5.9±0.1. A possible interpretation of these results is that the maintained SCC response to 5-HT is mediated by more than one receptor, one of which is 5-HT_4.

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350P ROLE OF CYCLOOXYGENASE IN TACHYPHYLAXIS TO THE VASOCONSTRICTOR EFFECTS OF ARACHIDONIC ACID IN THE PERFUSED KIDNEY OF THE RAT

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In the isolated perfused kidney of the rat, arachidonic acid (AA) elicits cyclooxygenase-dependent vasoconstriction that is mediated by prostaglandin endoperoxides (Quilley et al., 1989). The vasoconstrictor response to AA exhibits tachyphylaxis associated with diminished release of prostaglandins from the kidney (Quilley and McGiff, 1990). Tachyphylaxis does not involve the PGH_2/TxA_2 receptor as responses to the endoperoxide analogue, U46619, are unaffected by repeated administration of AA (Quilley and McGiff, 1990). These observations suggest that tachyphylaxis to the renal vasoconstrictor effect of AA involves inhibition/inactivation of cyclooxygenase. The studies presented here were undertaken to confirm this suggestion and address the mechanism involved.

Following pentobarbital (65 mg/kg) anesthesia, the right kidney was perfused in situ with oxygenated Krebs buffer at 37°C. The flow rate was adjusted to obtain a basal perfusion pressure (PP) of 80-110 mmHg. We measured increases in PP to 4 consecutive doses of AA (3 µg) given at 10 min intervals. In control rat kidneys (n=8) repeated administration of AA led to progressively smaller increases in PP, declining from 147±18 mmHg with the 1st dose to 8±4 mmHg with the 4th dose of AA ($p<0.01$); the respective increases in the release of 6-keto-$PGF_{1\alpha}$ were 4.1±0.9 ng/min and 0.2±0.1 ng/min ($p<0.01$). In another set of experiments (n=8) we sought to protect the cyclooxygenase against inactivation by infusing a reversible inhibitor, ibuprofen (5 µM), during the 2nd and 3rd doses of AA which then failed to elevate PP. Under these conditions, the 1st dose of AA elevated PP by 153±7 mmHg and the 4th dose by 99±22 mmHg compared to 8±4 mmHg in control kidneys ($p<0.01$); the increases in release of 6-keto-$PGF_{1\alpha}$ were 2.6+0.6 ng/min and 2.1±0.8 ng/min, respectively (N.S.). These results suggest that the conversion of AA by cyclooxygenase results in inhibition/inactivation of the enzyme. To address the role of free radicals in the reduced activity of cyclooxygenase the free radical scavengers, ascorbic acid (7.5 µM; n=5) and MK447 (2-aminomethyl-4-t-butyl-6-iodophenol; 20 µg/ml, n=4) were added to the perfusate and responses to repeated doses of AA (3 µg) tested. Both ascorbic acid and MK447 failed to prevent the development of tachyphylaxis to the vasoconstrictor response to AA.

In other experiments (n=7) we addressed the recovery of cyclooxygenase activity by allowing 30 min between the 3rd and 4th doses of AA. The 1st dose of AA elevated PP by 121±24 mmHg and the 4th dose by 56±23 mmHg compared to 8±4 mmHg when only 10 min elapsed between the 3rd and 4th doses of AA.

These results provide further evidence that tachyphylaxis to the vasoconstrictor effects of AA is due to reduced activity of cyclooxygenase which can be protected by ibuprofen. The results with ascorbic acid and MK447 do not support a role of free radicals in the reduced activity of cyclooxygenase.

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351P EFFECTS OF ELECTROCONVULSIVE SHOCK ON NORADRENALINE RELEASE FROM RAT FRONTAL CORTEX AND HIPPOCAMPUS

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Electroconvulsive shock (ECS) is an effective treatment for depression. The majority of biochemical and behaviourial evidence suggests that chronic treatment with some antidepressant drugs or ECS leads to downregulation of presynaptic α_2-adrenoceptors (Green, 1990). We have demonstrated that the extracellular concentration of NA in frontal cortex (FC) is regulated primarily by α_2 autoreceptors; while, in hippocampus (HI), transmitter uptake is predominant (Thomas & Holman, 1991). If the antidepressant effects of chronic ECS are due to changes in presynaptic α_2 autoreceptor, then following this treatment the effects on NA release should be greater in FC versus HI. The present study, used in vivo microdialysis to assess the effects of acute and chronic ECS on NA release and on responses to the α_2-adrenoceptor antagonist idazoxan.

Male Wistar rats (250-300g) were anaesthetised with chloral hydrate (400mg/kg, ip; supplement 90mg/kg) and concentric dialysis probes implanted into either the HI or FC. Samples (15min) were collected and analysed for NA content using HPLC-EC. Three baseline samples were collected following which animals were given either ECS or ear clipped alone (sham controls) and a further 5 samples collected. Chronically treated animals received ECS or ear clipping once daily for 7 days and on day 8 they were treated as above. The second group of chronic animals received 8 days of either ECS or ear clipping and during dialysis on day 9 were challenged with the α_2 antagonist idazoxan (10mg/kg, sc) after baseline samples were collected.

Basal NA concentrations in HI and FC were 4.5 ± 0.3 (n=5) and 4.1 ± 0.3 pg/sample (n=4) respectively. Acute ECS significantly increased NA release (HI 21.4 ± 1.2 (n=5); FC 11.6 ± 1.2 (n=4) pg/sample) in both brain regions as compared with sham controls. Chronic ECS did not change basal or ECS-stimulated NA release in HI. However, chronic ECS significantly elevated basal release (6.9 ± 1.0 pg/sample, n=4) in FC. ECS still stimulated NA release in FC and to the same extent as controls. In chronic ECS animals challenged with IDX, the responses in HI were the same as sham controls. In FC, basal release was again significantly elevated (5.5 ± 0.1 pg/sample, n=5) and the response to IDX was abolished as compared with controls.

These data show that chronic, but not acute ECS causes regionally specific increases in NA release. The elevated basal NA release and the lack of response to IDX in FC after chronic ECS is consistent with a downregulation of presynaptic α_2-adrenoceptors.

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352P [^{3}H]-IDAZOXAN BINDING TO α_2 ADRENOCEPTORS AND "NON-ADRENERGIC IDAZOXAN BINDING SITES" (NAIBS) IN THE RAT SPINAL CORD

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Recent studies (Boyajian et al., 1987a, b) have shown that idazoxan can bind to non-α_2 adrenergic sites in the brain and various peripheral nervous tissues. These sites have been referred to as "non-adrenergic idazoxan binding sites" or NAIBS. In this study we have investigated the binding of [^{3}H] idazoxan to membranes prepared from rat spinal cords to examine if NAIBS occur in this tissue. Membranes were prepared by centrifugation after homogenisation of whole spinal cords from 5 rats. They were frozen in aliquots at -80°C until used in experiments. Saturation and inhibition binding studies were done at 27°C in 50mM Tris-HCl buffer at pH 7.5. In the saturation experiments the membranes were incubated with [^{3}H] idazoxan at concentrations ranging from 0.1 to 60nM for 35 minutes for total binding and in the presence of 10µM unlabelled idazoxan for non-specific binding. These studies indicated a B_{max} value of 648±31 fmol/mg protein and a Kd value of 6.0±1.2nM for idazoxan binding to spinal cord membranes. These values are consistent with those found for idazoxan binding in rat brain (Brown et al., 1990). The competition binding studies utilised a concentration of 3nM [^{3}H] idazoxan with the following competing ligands over a maximum concentration range of 4 pM to 1mM, Idazoxan, RX821002 (a specific α_2 adrenoceptor ligand) from Reckitt & Colman Ltd. -Adrenaline from Sigma, and ±Rauwolscine from RBI. These studies showed that complete inhibition of [^{3}H] idazoxan was obtained with 10^{-5}M idazoxan with a Ki of 10.4±0.3nM. The percentages of non-displaceable [^{3}H] idazoxan binding found for the other competing ligands was 15% for -adrenaline, 40% for ±rauwolscine and 19% for RX821002 with concentrations up to 10^{-3}M, 10^{-3}M and 10^{-5}M respectively. The disparity in the percentages of non-displaceable [^{3}H] idazoxan binding could be due to displacement from more than one type of NAIBS. These findings however do suggest a consistent component of the [^{3}H] idazoxan binding in the rat spinal cord which is non-displaceable by the ligands used in these experiments. A definite function has yet to be established for NAIBS, but their existence in the spinal cord may be useful in the studies of the actions of idazoxan at the spinal level.

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353P α_1-ADRENOCEPTOR BINDING SITES IN POST-MORTEM BRAIN SAMPLES FROM ANTIDEPRESSANT-FREE SUICIDES AND CONTROLS

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Behavioural evidence suggests that α_1-adrenoceptor function is altered by antidepressants in animals (Maj et al., 1984), but there are few studies of the role of α_1-adrenoceptors in depression. We now report α_1- and α_{1A}-adrenoceptor binding in 9 brain regions from depressed suicides (15M, 7F; mean age ± SEM, 42±3 years; post-mortem delay ± SEM, 35±4 h) who had not recently received antidepressant drugs, and matched controls (15M, 7F; 43±3 years; 40±3 h). In addition the hemispheric distribution of α_1-adrenoceptors was studied in 3 brain regions in depressed suicides (n=9) and controls (n=9). α_1-Adrenoceptor binding sites were measured by saturation binding of [^{3}H]prazosin in well washed membranes (King et al., 1990).

The number or affinity of α_1- and α_{1A}-adrenoceptors did not differ significantly between right and left hemispheres in either suicides or controls. There were no significant differences in K_D or B_{max} values for total α_1- or α_{1A}-adrenoceptor binding sites in any region studied between depressed suicides and controls (Table 1), or when the suicides who died violently (n=12) or non-violently (n=10) were compared.

Table 1. B_{max} of α_1-adrenoceptor binding sites (fmol/mg protein, mean ± SEM, n=15-20).

Brain region	α_1-adrenoceptors		α_{1A}-adrenoceptors	
	Control	Suicide	Control	Suicide
Frontal cortex (Brod 11)	95 ± 6	80 ± 5	55 ± 3	44 ± 3
Temporal cortex (Brod 21/22)	115 ± 7	104 ± 8	71 ± 5	63 ± 5
Caudate	33 ± 1	33 ± 1	19 ± 1	18 ± 1
Hippocampus	234 ± 21	232 ± 20	170 ± 16	164 ± 15

The hemispheric distribution of α_1- and α_{1A}-adrenoceptors is symmetrical in both control and suicide brains. The number or affinity of α_1-adrenoceptors is not significantly altered in drug-free depressed suicide victims. Our findings are in contrast to Crow et al. (1984) who reported lower numbers of α_1-adrenoceptors in the hippocampus of suicides compared to controls.

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354P REGIONAL DIFFERENCES IN EVOKED DOPAMINE EFFLUX IN RAT ANTERIOR AND POSTERIOR CAUDATE PUTAMEN BRAIN SLICES

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Fast cyclic voltammetry (FCV) used with carbon fibre microelectrodes is an anatomically precise method for detecting electrically evoked dopamine (DA) efflux in brain slices (Palij *et al.*, 1990). Using FCV, we have observed a difference in the frequency characteristics of DA efflux evoked by trains of 25 pulses at 50 Hz in the anterior caudate putamen (aCPu; Bregma +1.00mm) and posterior caudate putamen (pCPu; Bregma -0.92mm), relative to single pulse efflux. The ratio (mean±sem) of such trains :single pulse varied at different recording sites from 1.47 ± 0.34 to 3.71 ± 1.61 in aCPu, and from 2.73 ± 0.60 to 9.40 ± 1.61 in pCPu ($n \geq 4$).

Further, in contrast to the aCPu, DA efflux in response to trains of 20 pulses in high ratio sites of pCPu showed a strong frequency-dependence and resembled that observed in the nucleus accumbens (ACb, Fig 1). The higher density of terminals from the A10 region in pCPu (Gerfen *et al.*, 1987), may be relevant since the ACb is also highly innervated from the A10 area.

The possible contribution by 5-hydroxytryptamine (5-HT) to the observed pattern of evoked efflux with frequency in pCPu, where striatal 5-HT concentrations are highest (Beal & Martin, 1985), seems unlikely since (a) the faradaic signal upon stimulation in the two regions was indistinguishable from exogenous dopamine, and (b) neither citalopram (1μM) nor fluvoxamine (1μM) increased peak efflux.

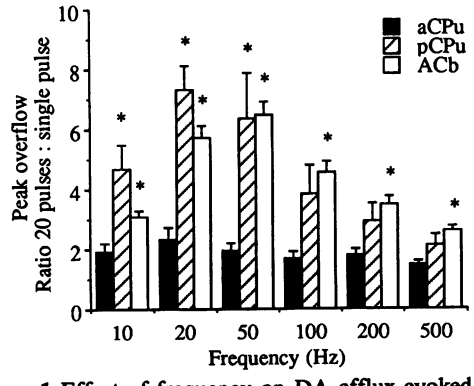

Figure 1 Effect of frequency on DA efflux evoked by 20 pulse trains in aCPu, pCPu & ACb. * $P<0.05$ cf. aCPu ($n \geq 5$).

In conclusion 5-HT does not appear to significantly contribute to the detectable frequency dependent pattern of transmitter efflux observed in pCPu. The factors controlling frequency dependent release under these conditions in pCPu may be a function of A10 innervation.

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355P COMPARISON OF THE STIMULATION-RELEASE CHARACTERISTICS OF STRIATAL AND LIMBIC DOPAMINE TERMINALS USING FAST CYCLIC VOLTAMMETRY IN BRAIN SLICES

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Fast cyclic voltammetry (FCV) at carbon fibre microelectrodes has been extensively used to study the stimulated efflux of dopamine (DA) in slices of rat forebrain nuclei. Most previous FCV studies have compared the pharmacology of DA release in limbic and striatal regions using a standard set of stimulus parameters (single 0.1ms pulses every 2min) although Trout *et al.* (1991) compared the frequency response characteristics of striatal and limbic DA terminals. In this study, we have examined the relative excitability of DA terminals in the striatum (CPu) and nucleus accumbens (Acb) to different stimulation pulse widths and currents.

All experiments were conducted in 350μm thick slices of Acb and CPu prepared from male Wistar rats (100-150g) as previously described (Palij *et al.* 1990). These were placed in a standard slice chamber and superfused with artificial cerebrospinal fluid (32°C, 1 ml/min). DA efflux was measured using FCV at carbon fibre microelectrodes implanted at a depth of 80μm below the surface of the slice. DA efflux was evoked using single constant-current rectangular pulses of variable width (5μs-5ms) and amplitude (10μA-10mA) applied at 5min intervals to a bipolar tungsten stimulating electrode positioned 200μm from the carbon fibre recording electrode.

In both nuclei, DA efflux increased with both pulse width and current up to the highest pulse width (5ms) and current (10mA) tested. DA efflux was abolished by tetrodotoxin (0.1μM), even on 5ms pulses, indicating that it was the result of action potentials rather than direct terminal depolarisation. Similarly, DA efflux was wholly calcium-dependent : replacement of Ca^{2+} with Mg^{2+} abolished DA efflux in both nuclei. DA terminals in Acb were significantly ($P < 0.05$ to 0.001) less able to release DA at short pulse widths than in CPu at all currents tested. At 10mA, the threshold pulse widths were 5μs (CPu) and 20μs (Acb). At lower current (0.1mA) the differences were even more pronounced, with thresholds of 0.1ms (CPu) and >5ms (Acb). The "standard" pulse width and current commonly used (0.1ms and 10mA) is 40-fold greater than threshold in CPu but only 5-fold greater in Acb.

These results are in sharp contrast with those of Yeomans *et al.* (1988) who reported that A9 and A10 excitability was comparable at the level of the axons in the median forebrain bundle. Thus it seems likely that the results of the present study reflect local differences either in excitability or in the DA release process at limbic and striatal terminals. Differences in stimulation-release characteristics between the two nuclei should be borne in mind when comparing apparent pharmacological differences in Acb and CPu.

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356P THE EFFECT OF A RETROGRADE NEUROTOXIN ON PROJECTIONS OF THE SUBSTANTIA NIGRA: HISTOLOGY AND DOPAMINE D_2 RECEPTOR BINDING

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Volkensin is a neurotoxic plant lectin which, when taken up by nerve terminals and retrogradely transported to the cell body, inhibits protein synthesis resulting in cell death, a process known as suicide transport (Stirpe et al., 1985). The neurotoxic properties of the proposed retrograde neurotoxin volkensin were investigated on the projections of the substantia nigra pars compacta (SNc) and substantia nigra pars reticulata (SNr). Eight male Sprague-Dawley (SD) rats received 3-5ng volkensin and 4 male SD rats received 3ng volkensin unilaterally into the striatum and tectum respectively. A recovery period of 5-7 days was allowed before sacrificing by barbiturate overdose. The brains were snap frozen in isopentane (-30°C). Twenty micron sections were cut on a cryostat and mounted onto subbed slides. Sections for histology were stained with cresyl violet. Sections for dopamine D2 receptor autoradiography were processed as previously described (Graham et al,. 1990), using the selective D2 receptor ligand [^{3}H]-sulpiride. Non-specific binding was defined using 1μM cold sulpiride. Cell counts were obtained using a microscope aided graticule.

Unilateral intrastriatal injections of volkensin caused a 60-79% decrease ($p<0.001$, paired students t-test) in SNc cell number on the ipsilateral as compared to the contralateral side. This decrease was associated with a 35-56% decrease ($p<0.05$, paired students t-test) in [^{3}H]-sulpiride binding to D2 receptors in the SNc. Animals with tectal injections showed gliosis and cell loss in the SNr.

The cell loss and decrease in D2 receptor binding, in the SNc after striatal injections, and cell loss and gliosis in the SNr after tectal injections, are attributed to the retrograde neurotoxic properties of volkensin. The peculiar qualities of volkensin make it of great potential use in defining more clearly specific neuronal pathways in the CNS.

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357P POTENTIATION OF COCAINE-INDUCED SPONTANEOUS LOCOMOTOR ACTIVITY AND STEREOTYPY BY HALOPERIDOL

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Cocaine augments locomotor activity and induces stereotypy (Scheel-Kruger et al 1977). Haloperidol can increase dopamine release by blockade of dopamine autoreceptors (eg Di Chiara 1990). In the present study we have investigated the effects of a low dose of haloperidol on cocaine induced locomotor activity and stereotypy.

Male Wistar rats (6 rats/treatment group) were administered either cocaine (15.0 mg/kg^{-1} s.c.) or saline (0.9%, 1.0 mls/kg^{-1} s.c.) 60 min after treatment with haloperidol (0.1 mg/kg i.p.) or saline. This treatment schedule was repeated 7, 14, 21 days later. Locomotor activity (photoelectric beam) and stereotypy (0-3 rating scale) were measured from 20 min after the injection of cocaine for 120 min. In a preliminary experiment haloperidol (0.1 and 0.5 mg/kg^{-1} i.p.) were compared for their ability to inhibit amphetamine (2.5 mg/kg^{-1} i.p.) induced locomotor activity.

Amphetamine hyperactivity was fully antagonised by the higher dose of haloperidol but was reduced only 27% by the lower dose. Cocaine (15 mg/kg^{-1} s.c.) significantly increased locomotor activity (+226% (day1) & 275% (day 7) $p<0.01$) and induced stereotypy (headshakes). Pretreatment with haloperidol (0.1 mg/kg^{-1} i.p.) non-significantly potentiated (+108%) the locomotor effects of cocaine after the first administration (day 1) and significantly (+206% $p<0.01$) after the second (day 7). After the third and fourth administrations there was no significant difference between the behavioural effects of cocaine with or without haloperidol pretreatment. The cocaine induced stereotypy was also potentiated by haloperidol on days 1 and 7 but not subsequently.

The results indicate that the low dose of haloperidol used may have predominantly antagonised pre-synaptic dopamine autoreceptors resulting in enhanced dopamine release, and thus potentiation of cocaine-induced locomotor activity and stereotypy. This effect was lost with repeated haloperidol administration as tolerance of the autoreceptors may develop (Di Chiara and Imperato, 1985).

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358P COMPARATIVE EFFECTS OF ANORECTIC COMPOUNDS ON FOOD INTAKE AND OVERT BEHAVIOUR IN RATS AND MICE

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An important factor in the study of anorectic compounds is that of dissociating primary anorectic effects from behavioural disruption, which can lead secondarily to decreases in food consumption. We have highlighted this problem by studying effects on behaviour and food consumption of several different compounds in two species, the mouse and rat. Following initial studies in mice we noted deviations from the expected results for fenfluramine and hence extended our investigations to compare directly the effects of fenfluramine in the mouse and rat. We have investigated the effects of diethylpropion and fenfluramine, clinically available anorectic drugs, and dexamphetamine, together with N-ethylnorephedrine on food intake, locomotor activity and overt behavioural effects in both mice and rats.

Female T/O strain mice or male Sprague-Dawley rats were used in these studies. Food intake was assessed in groups of 12 animals, which had been fasted overnight. Fifteen minutes after subcutaneous injection with the test compounds the animals were given access for 30 mins to a dish of weighed food (mash of equal parts water and pelletted mouse diet (CRM)). The average food intake was calculated, with allowance made for evaporation. Locomotor activity was assessed 30 mins after dosing for 6 mins using an automated open field. An Irwin-style primary observation test (Irwin, 1968) was carried out on the compounds during which overt behavioural effects were noted for 1 hour following subcutaneous administration.

The results were as follows: Overt effects = highest no-effect dose (mg/kg); Activity = dose required (mg/kg with 95% confidence limits) to increase locomotor activity to 200% of control or, (*), to decrease activity by 50%; Food Intake = dose required (mg/kg with 95% confidence limits) to decrease 30 min. intake by 50%.

	Mouse Overt effects	Mouse Activity	Mouse Food Intake	Rat Overt effects	Rat Activity	Rat Food Intake
dexamphetamine	1	2.1 (1.4-3.2)	0.5 (0.4-0.7)	2	2	0.37 (0.23-0.60)
diethylpropion	10	7.2 (5.2-9.5)	3.3 (2.0-4.9)	40	>16	3.7 (2.5-5.4)
N-ethylnorephedrine	40	No effect at 120	5.3 (3.7-7.5)	120	>250	11.7 (7.7-17.8)
fenfluramine	10	12* (9-20)	18 (10-30)	10	No effect at 3	1.0 (0.5-2.1)

Therefore fenfluramine, which is considered to act via serotonergic mechanisms (Garattini *et al.*, 1978) is a potent anorectic at doses which do not affect behaviour in the rat, while in the mouse there is no separation between anorectic and sedative doses. These data illustrate further differences in serotonin pharmacology between the two species, in addition to those reported recently with regard to thermoregulatory mechanisms (Bill et al., 1991). The anorectic compounds which are thought to act via noradrenergic and dopaminergic mechanisms, dexamphetamine and diethylpropion, show no differences between species. N-ethylnorephedrine is interesting in that it is anorectic in both species at doses substantially lower than those which affect activity and gross behaviour.

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359P THE EFFECT OF 8-OH-DPAT ON OPERANT FOOD INTAKE IN NON-DEPRIVED RATS

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8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) has been shown to increase food intake in non-deprived rats (Dourish et al., 1985). However, recent experiments have suggested that the drug elicits chewing or gnawing rather than eating (Montgomery, et al., 1988). The present study was undertaken to resolve this controversy. The effects of 8-OH-DPAT were investigated on food intake in non-deprived rats trained to make operant responses for food reinforcement. The use of operant paradigms eliminates any misinterpretation of results which could be caused by non-specific effects, such as drug-induced chewing, and also gives an indication of the motivation of the animal to work for food reinforcements (Baldwin et al., 1990). Male Wistar rats (n=7) were trained to press a lever in an operant conditioning chamber for food pellets on a fixed ratio of 6 (FR-6), as described previously (Ebenezer, 1985). Thereafter, the rats were fed ad libitum in their home cages, and given 6 further daily sessions in the operant chamber to press the lever for food on the FR-6 schedule. During the experimental sessions that followed, the non-deprived animals were injected s.c. with either physiological saline or 8-OH-DPAT (15.625 - 500 $\mu g\ kg^{-1}$) and placed separately in the operant chamber for 80 min. Operant food intake was measured during this period. Each rat received all doses of 8-OH-DPAT, and a wash-out period of 2-3 days was allowed between each dose. 8-OH-DPAT (15.625 - 500 $\mu g\ kg^{-1}$) produced significant effects on operant responding for food [$F(5,30) = 11.3305$, $P<0.01$]. The lower doses (i.e. 15.625, 31.25 and 67.5 $\mu g\ kg^{-1}$) caused significant increases in food intake ($P<0.05$, in each case). Thus, for example, 8-OH-DPAT (31.25 $\mu g\ kg^{-1}$) increased operant feeding by 225%. In contrast, the higher doses (i.e. 125 and 500 $\mu g\ kg^{-1}$) produced, in a dose-related manner, a number of stereotyped behaviours, such as flat body posture, wet dog shakes, and headweaving, which disrupted operant responding. There was an overall 45% reduction in operant feeding with the 500 $\mu g\ kg^{-1}$ dose, but this decrease was not statistically significant. The results of this study thus indicate that low doses of 8-OH-DPAT increase operant feeding in non-deprived rats, and provide support for the hypothesis that the action of this drug on feeding is not related to chewing or gnawing.

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360P THE EFFECT OF 5-HT ON VISUALLY-EVOKED AND NMDA-EVOKED RESPONSES IN THE RAT DORSOLATERAL GENICULATE NUCLEUS

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The thalamic dorsolateral geniculate nucleus (dLGN) relays visual information from the retina to the visual cortex. Retinogeniculate transmission is mediated by excitatory amino acid receptors in the cat (Sillito *et al*, 1990) and the rat (Crunelli *et al*, 1987), although the role of NMDA receptors in the rat dLGN remains unclear. Ionophoresis of 5-HT onto geniculate relay cells results in inhibition of spontaneous or electrically-evoked activity in rats (Rogawski and Aghajanian, 1980). The aim of this study was to examine the effect of serotonergic agents on both visually-evoked and NMDA-evoked responses in the rat dLGN.

Male Lister Hooded rats (200-250g) were anaesthetised with urethane (1.3-1.5 $g.kg^{-1}$, i.p.) and seven-barreled silicone-coated micropipettes were used to record extracellular neuronal discharge activity (Scott and Mason, 1991). Ionophoretic ejection of drugs was controlled by an electronic clock and retaining currents of ± 10nA were used between ejections with continuous automatic current balancing. The effect of ionophoresed 5-HT (10mM, 0-30nA), 5-CT (10mM, 0-10nA), 8-OHDPAT (10mM, 0-40nA) and (±)pindolol (10mM, 0-100nA) on visually-evoked activity and NMDA-evoked responses (10mM; 10s ejections (20nA) repeated at 120s intervals) in the dLGN was evaluated from integrated firing rate histograms. Ionophoresis of 5-HT, 5-CT and 8-OHDPAT in the dLGN resulted in a current -dependent, reversible inhibition of both the visually-evoked activity (mean ED_{50}: 5-HT 4.9 ± 1.2nA, n=9 cells; 5-CT -2.4 ± 0.7nA, n=8 cells; 8-OHDPAT 12.7 ± 4nA, n=4 cells), and NMDA-evoked responses in all cells studied (mean ED_{50}: 5-HT 7.3 ± 2.2nA, n=8 cells; 5-CT -1.2 ± 0.82nA, n=9 cells; 8-OHDPAT 8 ± 2.3nA, n=4 cells). Initial studies have shown that ionophoretic application of pindolol attenuated the inhibition of visually-evoked firing by 5-HT (mean ED_{50}: 45 ± 7.6nA, n=3 cells). Ionophoresis of 5-HT, 5-CT, 8-OHDPAT and pindolol had no effect on spike waveform.

The inhibition of visually-evoked and NMDA-evoked responses by 5-HT and 5-CT suggests that the inhibitory response may be mediated by a "5-HT_1-like" receptor. The 5-HT_{1A} receptor agonist 8-OHDPAT inhibits both the visually-evoked and NMDA-evoked responses and pindolol, a 5-HT_{1A} and 5-HT_{1B} receptor antagonist (Hoyer *et al*, 1985), attenuated the 5-HT inhibition of visually-evoked responses. These results suggest that the 5-HT-evoked inhibition of the visually-evoked and NMDA-evoked responses may be mediated through a 5-HT_{1A} receptor.

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361P 5-BENZYLOXYTRYPTAMINE IS A POTENT, BUT NON-SELECTIVE, AGONIST AT CENTRAL 5-HT$_{1D}$ RECEPTORS

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The benzylated derivative of 5-hydroxytryptamine, 5-benzyloxytryptamine (5-BT), has been reported to act at the 5-HT receptor subtype involved in the inhibition of neurogenic plasma extravasation in rat dura mater. The present study compares the 5-HT receptor affinities and efficacies of 5-BT with sumatriptan, a selective 5-HT$_1$-like receptor agonist, which is also active in the plasma extravasation model (Buzzi *et al.*, 1991).

In vitro radioligand binding assays were carried out as described in Table 1. Functional effects at 5-HT$_{1A}$ and 5-HT$_{1D}$ sites were determined by the inhibition of forskolin stimulated cyclase in guinea-pig hippocampus (DeVivo and Maayani, 1986) and substantia nigra (Waeber *et al.*, 1989) respectively and compared with their effects on the increase in phosphatidylinositol turnover in guinea-pig choroid plexus (5-HT$_{1C}$) Table 1 (Hoyer *et al.*, 1989).

Table 1

Receptor		pIC$_{50}$ (Radioligand Binding Studies)				pEC$_{50}$* (Functional Studies)	
	[^{3}H]Ligand (nM)	Tissue	Nonspecific (μM)	5-BT	Sumatriptan	5-BT	Sumatriptan
5-HT$_{1D}$	5-HT (1.5)	Pig Striatum	5-HT (10)	7.6	7.7	6.6	6.1
5-HT$_{1A}$	8-OHDPAT (1.5)	Pig Cortex	5-HT (10)	6.9	6.3	6.5	5.4
5-HT$_{1C}$	Mesulergine (1.5)	Pig Cortex	5-HT (10)	6.5	5.1	< 4.5	< 4.0
5-HT$_2$	DOB (0.4)	Rat Cortex	Cyproheptadine (1)	7.1	< 5.0	< 5.0	< 4.0
5-HT$_3$	Q-ICS205-930 (0.5)	Rat Cortex	MDL 72222 (10)	< 5.0	< 5.0	-	-

*Functional studies in guinea-pig brain. pIC$_{50}$ = -log$_{10}$ concentration of drug required to reduce specific binding by 50%. Data are mean of 3 or more separate experiments with SEM ≤ 5%.

In both receptor binding and functional studies, sumatriptan was found to be a potent 5-HT$_{1D}$ receptor agonist and was essentially inactive at all other 5-HT receptor subtypes tested with the exception of 5-HT$_{1A}$ where it displayed a moderate affinity (Table 1). 5-BT has a comparable affinity for the 5-HT$_{1D}$ site as sumatriptan, and is a potent agonist at this receptor, but also has actions at 5-HT$_{1A}$, 5-HT$_{1C}$ and 5-HT$_2$ receptors. Thus 5-BT lacks the selectivity of sumatriptan as a tool for the exploration of the functional effects of activation of 5-HT$_{1D}$ receptors in the CNS.

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362P 5-HT$_{1D}$ AND 5-HT$_{1E}$ BINDING SITES IN POST-MORTEM BRAIN SAMPLES FROM SUICIDES AND CONTROLS

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In the presence of drugs which fully occupy 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ binding sites, residual [^{3}H]5-HT binding is displaced by 5-carboxamidotryptamine (5-CT) with high and low affinity components that have been termed 5-HT$_{1D}$ and 5-HT$_{1E}$ sites respectively (Heuring & Peroutka, 1987; Leonhardt et al. 1989). We have now investigated the role of these binding sites in depressive illness.

5-HT$_{1D}$ and 5-HT$_{1E}$ binding sites were determined in post-mortem brain from 22 suicide victims, with a firm retrospective diagnosis of depression and who had not recently received antidepressant treatment and 19 matched controls. Saturation binding of [^{3}H]5-HT (0.5-32 nM, specific binding defined with 10 μM 5-HT) to total 5-HT$_1$, 5-HT$_{1D}$ and 5-HT$_{1E}$ sites were determined as previously described (Lowther et al., 1991).

Table 1 5HT$_{1D}$ and 5HT$_{1E}$ binding sites in depressed suicides

	Total 5-HT$_1$		5-HT$_{1D}$		5-HT$_{1E}$	
	C	S	C	S	C	S
Putamen	253±8	257±12	60±6	62±5	133±8	142±10
Globus pallidus	353±22	425±19*	221±17	274±18*	107±9	105±7
Frontal cortex	308±11	298±9	88±5	88±6	106±5	106±5
Parietal cortex	206±11	212±9	55±7	54±6	79±6	89±5

Bmax (fmol/mg protein) are means ± SEM, * p<0.05 Wilcoxon test.

In globus pallidus the number of 5-HT$_{1D}$ sites was significantly higher in suicides than controls (by 24%), whereas 5-HT$_{1E}$ sites did not differ (Table 1). The higher density of 5-HT$_{1D}$ sites was restricted to suicides who died violently (by 35%); non-violent suicides did not differ from controls. The density of 5-HT$_{1D}$ and 5-HT$_{1E}$ binding sites did not differ significantly between suicides and controls in frontal or parietal cortex or putamen, within the total suicide group or when divided by violence of death. These results suggest that 5-HT$_{1D}$ sites may be of aetiological significance in depression.

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363P BEHAVIOURAL EVIDENCE THAT 5-HT$_{1A}$ RECEPTOR MODULATION OF DOI HEAD-SHAKE IS PRESYNAPTIC

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A number of studies indicate the possibility of interactions between 5-HT$_{1A}$ and 5-HT$_2$ receptors (Heaton & Handley,1989 ; Arnt & Hyttel,1989 ; Yocca et al.,1990). We have therefore investigated the effects of the 5-HT$_{1A}$ ligands 8-OH-DPAT, gepirone, buspirone, ipsapirone (Hoyer,1988) and (±)pindolol (Yocca et al.,1990) on the head-shake tics (HSTs) induced by DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane) a selective 5-HT$_2$ / 5-HT$_{1C}$ agonist (Hoyer,1988).

Male MF1 mice (20-30g, N=6-11) received a submaximal dose of DOI (1.0 mg/kg, i.p) 10 minutes after i.p. pretreatments with 5-HT$_{1A}$ ligands (30 minutes for (±) pindolol; 24, 48, 72h after 3 doses of 300mg/kg for pCPA) and tic frequency was counted from videotapes for 6 minutes starting at 5 minutes after DOI injection.

DOI induced HSTs were reduced by 8-OH-DPAT (ID50: 0.014 [0.00035-0.51] mg/kg), gepirone (ID50: 0.54 [0.22-1.28] mg/kg), buspirone (ID50: 0.659 [0.206-2.086] mg/kg) and ipsapirone (ID50: 0.84 [0.19-3.72] mg/kg). Pindolol (5 mg/kg,i.p) also reduced DOI HSTs but abolished the inhibitory effect of 8-OH-DPAT (0.1 mg/kg), (% reductions: 8-OH-DPAT 75.3%, pindolol 38.8%, combination 32.9%, $F=7.91$, $df=16$, $p<0.05$). This pattern of effects suggests that pindolol was acting as a partial agonist at 5-HT$_{1A}$ receptors (Yocca et al., 1990). Pretreatment with pCPA did not affect DOI HSTs but antagonised the inhibitory effect of buspirone (1 mg/kg) ($F=11.64$, $df=40$, $p<0.01$). pCPA depleted whole brain 5-HT by 71.5 % and noradrenaline by 6.7%.

Thus all 5-HT$_{1A}$ ligands tested strongly inhibit 5-HT$_2$-receptor mediated HSTs. The antagonism of buspirone by pCPA suggests that 5-HT$_{1A}$ receptor involved is located presynaptically.

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364P THE EFFECTS OF WATER DEPRIVATION ON BRAIN 5-HT TURNOVER, PLASMA CORTICOSTERONE AND ELEVATED X-MAZE BEHAVIOUR

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A wide variety of stressors has been shown to elevate brain 5-HT turnover (Bliss et al., 1968; Knott and Curzon, 1974; Mitchell and Thomas, 1988), but there is no information on the effects of water deprivation on brain 5-HT turnover. A period of water deprivation is integral to the Vogel Conflict test, a widely used animal model of anxiety (Vogel, 1971). These experiments were therefore designed to investigate whether it is able to influence behaviour in another commonly used animal model of anxiety, the elevated X-maze (Handley and Mithani, 1984).

Groups of 6 naive male Wistar rats (150 - 230) were deprived of water for 12, 24, 36 and 48 hours and then killed. Plasma corticosterone was assayed by HPLC-UV and whole brain 5-HT and 5-HIAA by HPLC-EC. The ratio of 5-HIAA to 5-HT was calculated for each rat. Further groups of 6 rats were deprived for 48 hours or received ip saline or corticosterone 30 minutes prior to being placed on the maze and their behaviour was recorded on the video for 10 minutes.

12-48 hour water deprivation significantly elevated plasma corticosterone, although there was no clear relationship between this effect and the extent of deprivation. The 5-HIAA/5-HT ratio was significantly increased only at 36 and 48 hours. 48 hour water deprivation increased plasma corticosterone from 6.0 ± 0.8 to 19.9 ± 3.6 µg/dl ($p<0.01$), increased the 5-HIAA/5-HT ratio from 1.69 ± 0.09 to 2.48 ± 0.17 ($p<0.01$) and also increased the open/total arm entry ratio in the elevated X-maze from 0.18 ± 0.04 to 0.42 ± 0.05 ($p<0.01$) without significantly altering total entries. Corticosterone, 10 mg/kg, also elevated the entry ratio (from 0.30 ± 0.03 to 0.36) although total entries were increased.

These results confirm that water deprivation is capable of increasing plasma corticosterone and 5-HT turnover. Both water deprivation for 48 hours and administered corticosterone had anxiolytic-like effects in the elevated X-maze and the possibility that the effect of water deprivation was due to elevated plasma corticosterone is under investigation.

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365P ASSESSMENT OF ANXIOLYTIC AGENTS AND ONDANSETRON TO MODIFY EXTRACELLULAR LEVELS OF 5-HT IN THE RAT FRONTAL CORTEX

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There is increasing evidence to suggest that a reduction in central 5-hydroxytryptaminergic function results in an anxiolytic-like behaviour in preclinical models (for reviews see Chopin and Briley, 1987). In the present studies, we assess the ability of 3 pharmacological classes of compounds reported to have anxiolytic-like actions, to modify the in vivo release of 5-hydroxytryptamine from the frontal cortex of freely moving rats using the technique of microdialysis.

Microdialysis probes were implanted into chronically indwelling cannulae located in the frontal cortex (tip of probe A+0.7, V-7.0, L-1.5, relative to bregma at an angle of 45°) of male Lister Hooded rats (300-350g) 12 hours prior to the experiment. Probes were perfused with artifical CSF (composition, mM, NaCl 126.0, $NaHCO_3$ 27.40, KCl 2.40, KH_2PO_4 0.50, $CaCl_2.6H_2O$ 1.10, $MgCl_2.6H_2O$ 1.30, $NaHPO_4$ 0.49 and glucose 7.0, pH 7.4) at a rate of 2.0µl/min. 60 min later, 20 min samples were collected over a 7 hour period and analysed for 5-HT by HPLC with electrochemical detection. The 5-HT levels in the first 4 fractions constituted the basal release. After these collections (time = 0), the animals were treated with 0.9% w/v NaCl, 5.0mg kg^{-1} chlordiazepoxide, 4.0mg kg^{-1} buspirone or 10.0µg kg^{-1} ondansetron (all 1.0ml kg^{-1} i.p.).

As shown in Table 1, chlordiazepoxide and buspirone decreased the levels of extracellular levels of 5-HT by approximately 40-50% whilst ondansetron failed to modify the extracellular levels of 5-HT.

Table 1. The effect of anxiolytic agents and ondansetron on the extracellular level of 5-HT in the rat frontal cortex.

	Collection time (min)								
Treatment	0	20	40	60	80	100	120	140	340
0.9% NaCl	9.9±0.4	9.9±0.3	9.9±0.5	9.5±0.4	9.6±0.3	9.0±0.2	9.1±0.3	8.9±0.5	9.3±0.3
Chlordiazepoxide	9.1±0.4	8.8±0.2	8.2±0.9	5.3±1.0*	5.4±1.2*	10.2±2.0	7.1±1.5	8.7±2.0	8.3±1.5
Buspirone	9.8±1.0	8.0±1.0	8.2±1.6	6.3±1.1*	6.0±1.0*	5.7±1.0*	4.7±1.5*	5.2±1.5*	6.8±2.0
Ondansetron	9.0±0.1	9.2±0.5	8.8±0.4	9.0±0.3	9.6±0.5	9.1±0.4	8.7±0.4	9.1±0.2	8.7±0.2

Data represent mean ± SEM (pg/40µl sample), n = 5-8, *P<0.05, significant reduction compared to the basal level (one way ANOVA, followed by Dunnett's t test).

The present study confirms the finding that the $5\text{-}HT_{1A}$ receptor partial agonist buspirone reduces the extracellular level of 5-HT (Sharp et al., 1989). This action may account for its anxiolytic activity. The failure of the $5\text{-}HT_3$ receptor antagonist ondansetron to modify extracellular 5-HT levels suggests that this compound interacts with post-synaptic receptors with respect to the 5-HT neurone, thereby reducing the function of 5-HT.

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366P HEMISPHERIC ASYMMETRY OF [^{3}H]-IMIPRAMINE BINDING IN POST-MORTEM BRAIN SAMPLES FROM CONTROLS AND SUICIDES

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^{3}H-Imipramine (^{3}H-imip) is widely used to label 5-HT uptake sites. Marked hemispheric asymmetry in ^{3}H-imip binding sites (R > L) has been reported in samples of frontal cortex obtained at post-mortem from controls. Since this asymmetry was reversed (L > R) in subjects with psychiatric disorders (including suicides), it has been advocated that asymmetry of ^{3}H-imip binding is an indicator of psychiatric illness (Demeter et al., 1989). However ^{3}H-imip, as usually defined with desmethylimipramine (DMI) also labels sites unrelated to 5-HT uptake. Since we found no hemispheric asymmetry of ^{3}H-paroxetine binding in controls or depressed suicide victims (Lawrence et al., 1990a) the possibility that asymmetry of ^{3}H-imip binding is related to non-5-HT uptake sites has been studied. Saturation binding of ^{3}H-imip, defined with DMI and with Na^+ (which labels only 5-HT uptake sites, Hrdina, 1987) was measured concurrently in parietal cortex (Brodmann area 7), putamen and s.nigra from both hemispheres of 10 depressed suicides and 10 controls. Bmax values of Na^+ defined ^{3}H-imip binding were similar to those obtained with ^{3}H-paroxetine (Lawrence et al.,1990b) and there was no hemispheric asymmetry in controls or suicides (Table 1).

Table 1. Bmax of ^{3}H-imipramine binding defined with 100 µM DMI or Na^+ (fmol/mg protein).

	Controls				Suicides			
	DMI		Na^+		DMI		Na^+	
Hemisphere	Left	Right	Left	Right	Left	Right	Left	Right
Cortex (Ba7)	243 ± 28	268 ± 35	67 ± 10	72 ± 12	234 ± 20	239 ± 15	83 ± 9	78 ± 13
Putamen	557 ± 77	732 ± 94*	228 ± 28	242 ± 38	379 ± 45	455 ± 32	190 ± 19	214 ± 24
S.nigra	575 ± 52	552 ± 70	349 ± 25	314 ± 28	629 ± 81	579 ± 48	338 ± 41	314 ± 21

Means ± S.E.M. * significant differences between hemispheres, p > 0.05 paired t-test.

Bmax values for DMI defined ^{3}H-imip binding were substantially higher than for Na^+ defined ^{3}H-imip binding in each brain area. In the putamen there was a significant asymmetry (R > L) in controls and a similar trend in suicides. No significant asymmetry of DMI defined ^{3}H-imip binding was found in parietal cortex or s.nigra of controls or suicides. We conclude that hemispheric asymmetry in the putamen is related to differences in non-5-HT uptake sites labelled by ^{3}H-imip.

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367P THE EFFECT OF CENTRAL 5-HYDROXYTRYPTAMINERGIC LESIONS ON THE CEREBROVASCULAR RESPONSE TO HYPERCAPNIA

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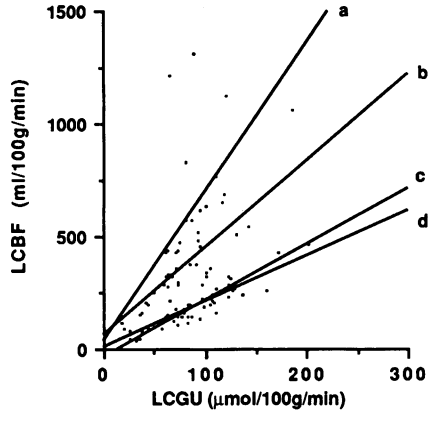
Serotonin-containing (5-HT) nerve fibres innervate cerebral blood vessels (Bonvento *et al.*, 1991), but the source of this innervation is controversal and no physiological role for perivascular 5-HT in the control of cerebral blood flow has yet been identified. The purpose of this study was to examine the effect of central 5-HT lesions upon the relationship between local cerebral blood flow (LCBF) and glucose utilization (LCGU) under both normo- and hypercapnic conditions.

Central 5-HT terminals were lesioned in male Sprague-Dawley rats (n=20) using methylenedioxyamphetamine as described by McBean et al.,(1991), controls receiving saline (n=20). Measurements of LCBF (n=20) and lCGU (n=20) were performed after 6 weeks, using [^{14}C]-iodoantipyrine and [^{14}C]-2-deoxyglucose autoradiography respectively (McBean *et al.*, 1991). Hypercapnia was induced with CO_2 in air, to give an arterial pCO_2 of 60mmHg in 10 LCBF and 10 LCGU experiments (5 lesioned in each group).

FIGURE 1



Over all of the 23 brain regions included in this study, there was a close coupling between flow and metabolism in all treatment groups ($r > 0.7$; Figure 1). As expected, hypercapnia induced global increases in LCBF in intact animals with little or no change in LCGU, thus resetting the relationship (m=3.87; Figure1b) compared to control (m=2.05; Figure 1d). Lesion alone had very little effect upon global flow-metabolism coupling (m=2.47; Figure 1c), but significantly enhanced the cerbrovascular response to hypercapnia (m=6.63; Figure 1a). Moreover, there was evidence of focal uncoupling by the lesion which was markedly enhanced in hypercapnia.

These results provide little evidence of a role for central 5-HT systems in the regulation of global cerebral blood flow. However, there may be a physiological role for the perivascular release of 5-HT from fibres of a central origin in regulating blood flow during periods of hypercapnic stress.

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368P A COMPARISON OF 5-HT BINDING IN THE SPINAL CORDS OF RATS AND SHEEP

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There have been reports of 5-hydroxytryptamine (5-HT) binding in rat spinal cord which suggest the existence of 5-HT_{1A+1B}, 5-HT_2 (Mitchell & Riley, 1985) and 5-HT_3 (Glaum & Anderson, 1988) receptor subtypes. In this study we have compared the binding of [^{3}H]5-HT, [^{3}H]spiperone and [^{3}H]GR65630 to membranes prepared from spinal cords of adult female Porton-derived Wistar rats and adult female sheep, using methods based on those described by Peroutka and Snyder (1979). Initial studies examined the saturation binding characteristics of [^{3}H]5-HT and showed that [^{3}H]5-HT bound to rat spinal cord membranes with a Kd of 1.80±0.11nM and a Bmax of 181.6±2.7 fmol.mg^{-1} protein; in the sheep these values were Kd=2.71±0.82nM and Bmax=440.1±42.1 fmol.mg^{-1}. Saturation studies using [^{3}H]spiperone gave Kd=3.01±0.79nM and Bmax=768.1±8.1 fmol.mg^{-1} in the rat; in the sheep these values were Kd=2.67±0.89nM and Bmax=353.8±43.7 fmol.mg^{-1}. [^{3}H]GR65630 also showed specific binding, with a Kd=1.67±0.44nM and Bmax=1193.4±161.4 fmol.mg^{-1} in the rat; Kd=1.53±0.29nM and Bmax=328.4±19.5 fmol.mg^{-1} in the sheep. Competition binding studies using 5-HT, RU24969, methysergide, 8-OH-DPAT, spiperone and GR38032F against 1nM [^{3}H]5-HT in both species suggested the existence of 5-HT_1, receptors, probably of the 5-HT_{1B} and 1A subtypes. IC_{50} values in the rat were: 5-HT=0.91nM, RU24969=5.01nM, methysergide=107.15nM, 8-OH-DPAT=63.10nM, spiperone=8511.4nM and GR38032F=31622.8nM; in the sheep these values were 6.92nM, 209.89nM, 10.96nM, 28.14nM, 38.02nM and 30199.5nM respectively. Competition studies using 5-HT, GR38032F and ICS205-930 against 0.4nM [^{3}H]GR65630 in the rat and 2nM [^{3}H]GR65630 in the sheep suggested the possible presence of non-5-HT_3 [^{3}H]GR65630 binding sites.

These findings suggested that 5-HT binding in rat and sheep spinal cord was similar for 5-HT_1 and 5-HT_2 receptor types, although the number of binding sites appeared to differ. The nature of [^{3}H]GR65630 binding is still being investigated.

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369P THE EFFECT OF MONOAMINE OXIDASE INHIBITORS ON DIMETHYLTRYPTAMINE-INDUCED HYPERACTIVITY IN MICE

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Mice pretreated with pargyline at a dose which inhibits both monoamine oxidase (MAO) isoenzymes, exhibited hyperactivity following systemic administration of N,N-dimethyltryptamine (DMT; Jenner et al, 1980; Morinan & Collier, 1981). In the present study, the effect of selective (clorgyline and selegiline) and non-selective (tranylcypromine) MAO inhibitors on DMT induced hyperactivity was investigated.

Male CFLP mice (25-35g) were injected (i.p.) with 0.9% saline (CON), or MAO inhibitors (all at 5mg/kg), 90 min before DMT 4mg/kg. The locomotor activity of pairs of mice was recorded (Panlab Actisystem) over the 3h period following the first injection.

Tranylcypromine and selegiline reduced activity during the first 90 min, but only in the former group was there evidence of DMT induced hyperactivity (Table 1). Tranylcypromine and clorgyline inhibited MAO-A activity by 95%, increased 5-HT concentration by 66%, and decreased 5-HIAA by 40-50% in the brain (Table 1).

Table 1. EFFECT OF MAO INHIBITORS ON BEHAVIOUR AND NEUROCHEMISTRY.

	Locomotor Activity 0-90 min	90-180 min	MAO Activity nmol/mg/h	5-HT ng/g	5-HIAA ng/g
CONTROL	2795 ± 446	190 ± 45	49.3 ± 4.2	292 ± 15	189 ± 4
TRANYLCYP.	1590 ± 327*	982 ± 305*	4.5 ± 2.0*	486 ± 17*	92 ± 9*
CLORGYLINE	2289 ± 393	283 ± 49	5.2 ± 0.7*	484 ± 26*	120 ± 8*
SELEGILINE	1211 ± 105*	377 ± 95	49.6 ± 11.1	289 ± 13	187 ± 10

Each value represents the mean ± s.e.m. of n=7 (columns 1 & 2) or n=6 (columns 3,4 & 5). *P < 0.05(vs. CON).

These data show that both MAO-A and -B must be inhibited before DMT induced hyperactivity is observed. A similar effect of selective MAO inhibitors on l-tryptophan induced behaviour has also been reported by Sleight et al (1988).

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370P STRUCTURAL REQUIREMENTS FOR HIGH BINDING AFFINITY TO THE 5-HT TRANSPORTER FOR β-CARBOLINES

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Specific 5-HT uptake inhibitors are known to bind to the 5-HT transporter with high affinity (Raisman *et al.*, 1979). Both [^{3}H]-citalopram and [^{3}H]-imipramine can be displaced from their binding sites at the 5-HT transporter by β-carbolines, especially by an endogenous one, pinoline (6-methoxytetrahydro-β-carboline). The binding affinity studies were based on the inhibition of [^{3}H]-imipramine binding in chicken retina (Kari *et al.*, 1988) and [^{3}H]-citalopram binding in human platelets. Structures of the 5-HT uptake inhibitors were calculated using the semi-empirical molecular orbital package MOPAC at the AM1 level. Molecular electrostatic potentials were calculated with the CHEMX software using the VSS program. Conformation analyses were made using molecular mechanics and the CHARM force field. Based on both electronic and steric properties a QSAR study was developed using eight tetrahydro-β-carbolines for [^{3}H]-imipramine binding (James, 1988). Mapping of the binding site was based on the superimposition of the pharmacophore of several specific 5-HT uptake inhibitors including citalopram and imipramine (Marshall, 1987). Pinoline had the highest binding affinity to [^{3}H]-citalopram and [^{3}H]-imipramine binding sites of all the compounds tested. Also according to the QSAR study pinoline was predicted to have the highest affinity to the binding site. The methoxy group at the C-6 position of the β-carboline structure seemed essential for high affinity. Further substitution, substituents at other positions or dehydrogenation reduced affinity. However, the binding affinity of 1,2-dehydro-pinoline to the [^{3}H]-citalopram binding site in human platelets was half that of pinoline, the IC_{50} values were 110 nM and 50 nM, respectively. Pinoline as well as the other β-carbolines did not fit perfectly to the model of the binding site. This model includes two hydrophobic pockets, an anionic site and a couple of sites for electrostatic interactions. β-carbolines can interact with this binding site by the protonated nitrogen of the tetrahydropyridine ring and by the electrostatic properties. They can occupy only one of the two hydrophobic pockets because of their rigid ring structure. This may result in different binding mechanisms and reduced binding affinity in comparison to citalopram. It seems that both steric and electronic properties of β-carbolines determine their binding affinity to the 5-HT transporter.

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371P THE EFFECT OF DEXAMETHASONE, ALONE OR IN COMBINATION WITH GRANISETRON, ON CISPLATIN-INDUCED EMESIS IN THE FERRET

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Dexamethasone is often given in combination with other anti-emetic agents. It has recently been shown to increase the anti-emetic activity of a submaximal dose of ondansetron (GR 38032F) against cytotoxic agents in both ferret and man (Cunningham et al, 1989; Hawthorn and Cunningham, 1990). In this study, the effect of dexamethasone (D) either alone or in combination with granisetron (G; BRL 43694) has been examined against cisplatin-induced emesis in the ferret. Intravenously cannulated male ferrets (1.16-1.95 Kg; fitch or albino) were challenged with cisplatin (10 mg/kg i.v.) and monitored for 240 min from vomiting and associated behaviours. D (1.0-5.0 mg/kg p.o.) was dosed 60 min before, and G (0.005-0.5 mg/kg i.v.) was dosed 15 min before cisplatin. The vehicle, methyl cellulose (M; p.o.), or saline (S; i.v.) was dosed at the same time as appropriate. Results are given as mean±SEM and compared to controls using Student's 't' test.

Effect of dexamethasone ± granisetron against cisplatin-induced emesis

Treatment	LP (min)	V	R	Bu	Ba	N vomiting/N tested
M + S	68±6.3	16±1.4	113±0.1	14±3.4	5.6±0.9	5/5
D (5.0) + S	170±42*	3.3±2.1***	29.3±18.0**	2.0±1.7*	1.3±0.9**	2/4
D (2.5) + S	96±14.5	3.3±1.0***	32.5±7.1***	5.8±3.4	0.8±0.5**	4/4
D (1.0) + S	96±8.5*	7.0±2.3**	65.5±18.4*	5.5±0.9	3.8±1.7	4/4
D (2.5) + G (0.5)	240***	0***	0***	0***	0***	0/4
D (2.5) + G (0.05)	221±19***	0.5±0.5***[a]	3.3±3.3***[a]	0.8±0.8**	0.5±0.5**	1/4
D (2.5) + G (0.005)	180.0±34.9**[a]	1.0±0.7***	62.3±27.0	7.0±2.7	1.8±0.8*	2/4
G (0.5)	240***	0***	0***	1.5±1.3***	0.2±0.2*	0/6
G (0.05)	159.5±20.5***	4.7±1.3*	73.2±16.8	10.7±5.8	2.6±0.9	5/6
G (0.005)	86.5±8.9	8.8±2.9	82.5±10.7	10.7±3.5	4.3±1.5	6/6

*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$ versus control a=$p \leq 0.05$, v. granisetron alone. ( ) dose in brackets, mg/kg. If no vomiting occurred, LP = 240 min. LP = latency to vomit; no. of vomits (V); retches (R); burrowing (Bu) or backing (Ba) episodes.

Dexamethasone was able to increase in an additive manner the anti-emetic activity of submaximal doses of granisetron against cisplatin; there was no evidence of synergy between D and G, as has been claimed between D and ondansetron (Hawthorn and Cunningham, 1990). However, at a dose of 0.5 mg/kg i.v., granisetron alone was able to completely protect against the emetic effects of cisplatin.

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372P INHIBITION OF [^{3}H]-5–HT UPTAKE AND [^{3}H]-PAROXETINE BINDING IN RAT HIPPOCAMPUS BY THE POTASSIUM CHANNEL BLOCKERS QUININE AND QUINIDINE

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A recent study showed that treatment of rats with quinine following preliminary administration of the monoamine oxidase inhibitor tranylcypromine gave rise to the classical 5HT behavioural syndrome (Wang et al, 1990). Quinine is reported to block potassium ion channels and may therefore produce this effect by blocking the potassium channels on serotonergic nerve terminals, depolarising them and so inducing 5HT release. We have therefore studied the effects of quinine, and its stereoisomer quinidine, on the release of ^{3}H-5HT from preloaded rat hippocampal synaptosomes and also their potency to block the active reuptake of ^{3}H-5HT and binding of ^{3}H-paroxetine in a similar preparation.

Synaptosomes were prepared from the hippocampus of male Sprague-Dawley rats (200g) For 5HT release studies, the synaptosomes were first incubated with ^{3}H-5HT (30nM), washed by repeated centrifugation and then release was induced by exposure to quinine or quinidine. Efflux of ^{3}H-5HT was estimated as percent of total ^{3}H-5HT. Uptake of ^{3}H-5HT (30nM) was measured according to the method of Wood & Wyllie (1981). Binding of ^{3}H-paroxetine (0.5nM) to hippocampal membranes was determined according to the method of Mellerup & Plenge (1986). Dose-response data were analysed using a non-linear regression program.

Table 1: Affinity (μM) of quinine and quinidine to induce ^{3}H-5HT release (EC_{50}) and inhibit ^{3}H-5HT uptake (IC_{50}) and ^{3}H-paroxetine binding (IC_{50}) in rat hippocampus. Values represent mean ± sem ; n=3.

	^{3}H-5HT Release	^{3}H-5HT Uptake	^{3}H-Paroxetine Binding
	EC_{50}	IC_{50}	IC_{50}
Quinine	180 ± 15	30 ± 3	45 ± 7
Quinidine	70 ± 11	0.8 ± 0.1	0.9 ± 0.1

As shown in Table 1, quinine and quinidine proved weak inducers of ^{3}H-5HT release with little difference in potency. By comparison they were substantially more potent inhibitors of ^{3}H-5HT uptake, quinidine being significantly more potent than quinine ($p<0.001$; unpaired t-test). Their affinity to block ^{3}H-5HT uptake corresponded closely with their potency to inhibit ^{3}H-paroxetine binding.

The lack of correlation between the potency of quinine and quinidine in inducing ^{3}H-5HT release and inhibiting ^{3}H-5HT uptake in hippocampal synatosomes suggests that these effects are mediated by two distinct mechanisms. The close correlation between their potency to block ^{3}H-5HT uptake and to inhibit ^{3}H-paroxetine binding suggests that they bind to the 5HT transport protein and hence reduce 5HT uptake. Hence the development of the 5HT behavioural syndrome in rats following their treatment with tranylcypromine and then quinine may be partly due to blockade of 5HT reuptake.

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373P N-ETHOXYCARBONYL-2-ETHOXY-1,2-DIHYDROQUINOLINE (EEDQ) DOES NOT INTERACT WITH RAT BRAIN 5-HT$_3$ RECEPTORS *IN VIVO*

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Binding studies have shown that the peptide coupling agent, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), irreversibly interacts with several types of neurotransmitter receptor including dopamine D1 and D2, 5-HT2, some 5-HT1 and α-adrenergic receptors (Meller *et al.*, 1985). However, not all receptors are susceptible; β-adrenergic, muscarinic cholinergic, opiate and GABA receptors are unaffected by EEDQ pretreatment (Meller *et al.*, 1985). In view of the above, we have now examined whether EEDQ administration inactivates 5-HT3 receptors as determined by *ex vivo* measurement of [3H]-GR65630 binding to rat entorhinal cortex.

Male CD rats (200-250g; Charles River), received EEDQ (6 mg/kg) or vehicle i.p. Twenty-four hours later, samples of striatum (for D1 and D2 receptors), frontal and entorhinal cortex (for 5-HT2 and 5-HT3 receptors, respectively) were taken from each animal and stored at -80°C. All tissues were used within 24h. Six-point saturation binding assays were performed on tissue from each animal using [3H]–SCH 23390 (0.06-2nM defined by 100μM chlorpromazine) for D1 , [3H]-spiperone (0.008-1nM defined by 10μM sulpiride) for D2, [3H]-ketanserin (0.06-2nM defined by 5 μM methysergide) for 5-HT2 and [3H]-GR65630 (0.06-2nM defined by 30μM metoclopramide) for 5-HT3. Receptor number and affinity were determined by non-linear curve fitting.

Table 1 Effect of EEDQ administration on dopamine and 5-HT receptor subtypes in rat brain.

Treatment	D1		D2		5-HT2		5-HT3	
	Bmax	Kd	**Bmax**	Kd	**Bmax**	Kd	**Bmax**	Kd
Control	**82.3 ± 4.78**	0.34 ± 0.20	**18.0 ± 0.81**	0.05 ± 0.006	**14.15 ± 1.93**	0.63 ± 0.07	**1.70 ± 0.05**	0.49 ± 0.05
EEDQ	**30.0 ± 2.39*****	0.34 ± 0.01	**6.3 ± 0.88*****	0.05 ± 0.006	**3.95 ± 0.58*****	0.48 ± 0.06	**1.98 ± 0.19**	0.41 ± 0.04

Bmax (fmol/mg wet wt.) and Kd (nM) values are mean ± s.e. mean (n=6-10) ; *** $p < 0.001$ compared with control (Student's t -test).

This study clearly demonstrates that EEDQ does not inactivate 5-HT3 receptors, in addition to confirming the findings of Meller *et al.* (1985) regarding the inactivation of D1, D2 and 5-HT2 receptors. Receptor affinities were not altered by EEDQ treatment. Belleau *et al.* (1969) suggested that EEDQ inactivates receptors by interacting with a carboxyl group to create a mixed carbonic anhydride; this reactive group then alkylates a nucleophilic site in the receptor. Thus only those receptors with a carboxyl group in or close to the binding site are inactivated by EEDQ. It can be inferred from these experiments that unlike other 5-HT receptors (5-HT2, some 5-HT1), 5-HT3 receptors are unlikely to possess a carboxyl group closely associated with the ligand binding site.

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cortical 5-HT$_3$ receptor recognition site density

374P EFFECTS OF CHRONIC PARA-CHLOROPHENYLALANINE AND 8-OH-DPAT ADMINISTRATION ON RAT CORTICAL 5-HT$_3$ RECEPTOR RECOGNITION SITE DENSITY

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We have previously demonstrated that the chronic administration of the 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OHDPAT) but not paroxetine reduced the density of 5-HT$_3$ receptor recognition sites (Barnes *et al*, 1991). Such treatments would be expected to decrease and increase extracellular 5-hydroxytryptamine (5-HT) levels, respectively (Sharp *et al*, 1989). Consequently, we now report the effect of chronic administration of the tryptophan hydroxylase inhibitor para-chlorophenylalanine (pCPA) on the density of 5-HT$_3$ receptor recognition sites within the rat cortex.

Rats (female, hooded-Lister, Bradford University bred; allowed access to food and water 'ad libitum') were treated daily with 8-OHDPAT (1.0mg kg^{-1} i.p.), pCPA (200mg kg^{-1} i.p.), vehicle (5.0% v/v polyethylene glycol 400, 1.0ml kg^{-1} i.p.) or pCPA and 8-OHDPAT (200mg and 1mg kg^{-1} i.p. respectively) for 28 days, pCPA treatment starting 4 days prior to 8-OHDPAT treatment. Animals were killed and the cortex dissected and frozen. 5-HT$_3$ receptor recognition site density was determined by saturation experiments (minimum 6 concentrations, range 0.075-4.0nM) using [^{3}H]GR65630 (specific activity 84.2Ci mmol^{-1}, NEN) according to the method of Barnes and colleagues (1991). Non-specific binding was defined by the inclusion of granisetron (10μM).

Chronic administration of 8-OHDPAT significantly reduced the density of 5-HT$_3$ receptor recognition sites within the rat cortex compared to vehicle treated animals, whilst chronic pCPA or pCPA plus 8-OHDPAT had no effect (B_{max}; 50±6, 34±4, 54±12 and 48±5fmol mg^{-1} protein, mean ± s.e.m., n = 6-7, vehicle, 8-OHDPAT, pCPA and pCPA plus 8-OHDPAT respectively; difference between vehicle and 8-OHDPAT treated animals $P<0.05$, Student's t-test). No treatment significantly altered the affinity of the radioligand for the 5-HT$_3$ receptor recognition site (K_D; 0.81±0.13, 0.56±0.15, 0.75±0.18, 0.74±0.11nM, mean ± s.e.m., n = 6-7 vehicle, 8-OHDPAT, pCPA and combined treatments respectively).

In the present study we have confirmed that chronic 5-HT$_{1A}$ receptor stimulation decreases the density of cortical 5-HT$_3$ receptor recognition sites. Whilst inhibition of the synthesis of 5-HT using pCPA failed to modify the density of 5-HT$_3$ recognition sites, such a treatment was shown to abolish the effects of 8-OHDPAT.

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375P 5-HT ANTAGONISTS: ICS-205930 AND MDL-72222 INDUCE ANTINOCICEPTION BY CHOLINERGIC ACTIVATION

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It has been reported (Bartolini et al., 1989; Gualtieri et al., 1989; Iwamoto 1989; Bartolini et al. 1990; Ghelardini et al., 1990) that a significant antinociception in rodents can be induced by selective antagonists of both muscarinic autoreceptors (atropine at very low doses, AFDX-116 and methoctramine) and dopaminergic heteroreceptors located on cholinergic terminals (haloperidol, raclopride). This antinociceptive effect could be due to an increase of ACh release, since pretreatment of animals with hemicholinium-3 (HC-3) and vesamicol prevented such effects. 5-HT_3 antagonists have been shown to antagonize the inhibitory effect of 2-methyl-5-HT on ACh release (Barnes et al., 1989); we therefore thought it of interest to investigate whether the two selective 5-HT_3 antagonists, ICS-205930 and MDL-72222, were able to induce antinociception in laboratory animals. Experiments were performed in mice and rats by using three different noxious stimuli: chemical (writhing test), thermic (hot-plate test) and mechanical (paw-pressure test) .

The present results show that either ICS-205930 (20 mg kg^{-1} s.c.) or MDL-72222 (25 mg kg^{-1} s.c.) produce, 15 min after their administration, a significant increase of pain threshold either in hot-plate test (+56% and+85% respectively) or in paw-pressure test (+109% and +123%). In agreement with the general observation that antinociception is obtainable at lower doses when the writhing test is used, ICS-205930 was found to significantly reduce (-63%) the number of writhes at a dose (5 mg kg^{-1} s.c.) which was ineffective in the hot-plate test. ICS-205930 and MDL-72222 exerted their antinociceptive effect without affecting the normal behaviour of the animals. The site of the antinociceptive effect of ICS-205930 and MDL-72222 is located in the CNS since a statistically significant antinociception using the hot-plate and the writhing test was observed following i.c.v. treatment (25-50 µg per mouse). The antinociception induced by 5-HT_3 antagonists was prevented by pretreatment with the following anticholinergic drugs: atropine (5 mgkg^{-1} i.p.), dicyclomine (l0 mg kg^{-1} i.p.) and HC-3 (1 µg per mouse i.c.v.) administered 30 min, 25 min and 5 h respectively before test. On the other hand naloxone (l mg kg^{-1} i.p. and reserpine (2 mg kg^{-1} i.p.) did not modify the action of 5-HT_3 antagonists. These results suggest an involvement of the central cholinergic system in the antinociceptive effect of ICS-205930 and MDL-72222.

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376P CHANGES IN THE EFFECTS OF NMDA UPON SPONTANEOUS DORSAL ROOT ACTIVITY WITH POSTNATAL DEVELOPMENT, IN ISOLATED HAMSTER SPINAL CORD

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Isolated mammalian spinal cords maintained at 25-27°C, *in vitro,* show spontaneous bursts of action potentials emerging from the cord in the dorsal roots. Changes in the frequency and pattern of this activity during the first 8 weeks of post-natal life have been reported (Abdul Razzak *et al.,* 1990). The adult pattern of spontaneous dorsal root activity has been shown to be insensitive to inclusion of 1mM Mg^{2+} or 5µM AP5 in the bathing medium, suggesting that NMDA receptors are not involved in the generation of spontaneous dorsal root activity in adults (Bagust *et al.*,1989). In cords taken from young animals the spontaneous activity in the dorsal roots is depressed by both magnesium and AP5, indicating an involvement of NMDA receptors (Abdul Razzak *et al.,* 1991). Investigation of the effects of directly applied NMDA to isolated hamster cords has demonstrated changes in sensitivity during post-natal development.

Experiments were performed on whole isolated spinal cords taken from golden hamsters (*Mesocricetus auratus)* between one and six weeks after birth. Spontaneous activity was recorded from lumbar dorsal roots using silver wire electrodes, or from the dorsal horn of the lumbar spinal cord using low resistance (5MΩ) glass microelectrodes. Cords were bathed in Mg^{2+} free medium, and the effects of addition of NMDA for 10 minutes, upon the frequency and pattern of spontaneous activity studied.

Activity recorded from 5-6 week old animals consisted of bursts of action potentials characteristic of spontaneous activity recorded in adult animals. The frequency of this activity was depressed to 66.4% (s.e.mean ± 3.0, n=13) of the control frequency by the inclusion of 10µM NMDA in the bathing medium. In the second postnatal week cords bathed in Mg^{2+} free medium showed spontaneous activity which consisted of single spikes firing at a mean frequency of 21.7Hz (s.e.mean ± 3.5, n=12). Addition of 10µM NMDA to the bathing medium produced a complex sequence of changes in the firing frequency. Recordings were made from a total of 12 dorsal horn sites in 6 cords. The predominant pattern was of a short latency excitation, with an increase of the mean firing frequency to 156% above the control level (s.e.mean ± 29.0, n=9), which lasted for between 1 and 5 minutes, and was followed by a profound decrease in the firing rate to 23.2% of the control (s.e.mean ± 7.7, n=9), which persisted for the remainder of the 10 minute period. Activity returned to control levels within 30minutes of washing. In one case 10µM NMDA produced a sustained excitation which lasted for the entire 10 minute exposure to NMDA, and in another 2 cases only depression was produced.

10µM NMDA had no effect upon the pattern of the spontaneous activity, the neonatal cords continued to produce single spikes, and cords from more mature animals bursts of action potentials.

These results confirm that the mechanism that is responsible for generating spontaneous activity in the dorsal horn of the isolated hamster spinal cord is sensitive to NMDA in the neo-natal animal, but this sensitivity is lost as the cord matures.

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377P ACTIONS OF THE FOUR ISOMERS OF 1-AMINOCYCLOPENTANE-1,3-DICARBOXYLATE (ACPD) IN THE HEMISECTED ISOLATED SPINAL CORD OF THE NEONATAL RAT

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We have examined the actions of the four stereoisomers of ACPD, rigorously purified to minimize enantiomeric cross contamination, on motoneurones in the hemisected isolated spinal cord preparation from 1-5-day old rats. The substances were synthesized by modification of the method of Curry *et al* (1988) and purified to a level of > 98% stereoisomeric purity as determined by chiral HPLC. Structures were checked by X-ray crystallography. Recordings were made from ventral roots of motoneuronal potentials evoked by dorsal root stimulation (DR-VRP) or induced by amino acids added to the medium.

1R,3R-ACPD (1-3 μM) produced depolarizing responses that were similar in potency and in sensitivity to blockade by D-2-amino-5-phosphonopentanoate (D-AP5, 25 μM) to those produced by N-methyl-D-aspartate (NMDA,1-3 μM).

1S,3R-ACPD (2-100 μM) produced depolarizing responses which tended to fade during continued administration of the substance or on repeated dosing. In a tetrodotoxin (0.1 μM)-blocked cord, depolarizations produced by 1S,3R-ACPD (100 μM) were not blocked by D-AP5 (25 μM) or by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) which abolished responses to α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA).These responses of 1S,3R-ACPD may reflect activity of the metabotropic receptor (Irving *et al*, 1990).

1S,3S- and 1R,3S-ACPD (50-500 μM), produced depolarizations corresponding in potency and in antagonist sensitivity with their contents of approximately 1% in each case of 1R,3R- and 1S,3R-ACPD, respectively. Any intrinsic depolarizing activity of 1S,3S- and 1R,3S-ACPD is likely to be low.

At low concentrations (1-10 μM) 1S,3S-ACPD and 1S,3R-ACPD depressed synaptic responses (particularly the fast, monosynaptic component of the DR-VRP measured in the presence of 1-2 mM Mg^{2+}/25-50 μM D-AP5) without antagonizing depolarizations produced by L-glutamate, NMDA, AMPA or domoate. It is suggested that these synaptic depressant effects are presynaptic in origin.

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378P POTENCY AND SPECIFICITY OF GLYCINE-SITE N-METHYL-D-ASPARTATE (NMDA) ANTAGONISTS ON RAT SPINAL CORD *IN VITRO*

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The NMDA receptor-channel complex is characterized by a glycine regulatory site at which many non-competitive NMDA antagonists act by displacing endogenous glycine. Their action can be reversed by glycine or D-serine (Fletcher *et al.*, 1990). It has been recently shown that, in rat hippocampal slices, mephenesin reduced the number of spontaneous spikes occurring in Mg^{2+}-free medium. D-serine partially reversed this effect, suggesting that mephenesin was a glycine-site NMDA antagonist (Shuker *et al.*, 1991). The aim of the present study was to characterize the potency, specificity and reversibility of mephenesin and other recently claimed glycine antagonists (Leeson *et al.*, 1991) using the grease seal technique applied to rat spinal cord. Briefly, the spinal cords were dissected from freshly killed 2-7 days old rats, hemisected and placed in a two compartment Perspex chamber so that a greased barrier fitted transversely across the thoraco-lumbar cord. DC potentials were recorded along the caudo-cranial axis in response to superfusion of the lumbar cord by excitatory amino acids which were added in 5 ml aliquots.

Some antagonists fully suppressed the response of spinal cord to 20 μM NMDA, the rank order of potency being 5,7-dichlorokynurenate (IC_{50}=4.0 μM)>6,8 dinitroquinoxaline-2,3-dione (MNQX)> (7.7 μM)>7-chlorokynurenate (19.3 μM). On the other hand 5-chloroindole-2-carboxylate and 1-hydroxy-3-aminopyrrolid-2-one (HA-966) were partial antagonists (IC_{50}> 100 μM). Of the above, only MNQX blocked the response to 20 μM α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) with an IC_{50} of 15.5 μM. Mephenesin was a weak NMDA antagonist (IC_{50} about 7 mM) with non-selective effects on the AMPA response at concentrations above 0.32 mM. D-serine (100 μM) in the absence of antagonist did not potentiate the responses to NMDA. On the other hand 100 μM D-serine partially reversed the inhibition of the NMDA response by the above antagonists except mephenesin.

In conclusion, the lack of effect of D-serine alone suggests that in our experimental conditions glycine sites on spinal neurons are occupied by an endogenous ligand during the application of NMDA. The present study also confirms that 5,7-dichlorokynurenate is one of the most potent and specific glycine-site antagonists, while on the contrary, mephenesin does not show the profile of a glycine antagonist.

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379P EXCITATION OF RAT THALAMIC NEURONES *IN VIVO* BY THE EXCITATORY AMINO ACID, *trans*-(±)-1-AMINO-CYCLPENTYL-1,3-DICARBOXYLATE (*t*-ACPD)

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We have previously shown the involvement of excitatory amino acid receptors of both the N-methyl-D-aspartate (NMDA) type and the non-NMDA type (sensitive to 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)) in synaptic responses of ventrobasal thalamus (VB) neurones (Salt & Eaton, 1991). There is now good evidence to suggest that there is a further excitatory amino acid receptor which can be acted upon by quisqualate and other agonists: the so-called metabotropic glutamate receptor, for which *t*-ACPD may be a selective agonist (Schoepp *et al.*, 1990). We have investigated the actions of *t*-ACPD on VB neurones in rats anaesthetised with urethane. Extracellular action potential recordings and iontophoretic drug applications were made with multibarrel glass electrodes.

t-ACPD was applied to more than sixty VB neurones and in all cases the agonist produced excitations which were slower in onset and of longer duration than those produced by the agonists NMA, kainate or (R,S)-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA). Responses to *t*-ACPD were resistant to the non-NMDA receptor antagonist, CNQX (responses 122% of control (±9.6 s.e.m., n=15)), and to the NMDA receptor antagonist 3-((R,S)-2-carboxy-piperazin-4-yl)-propyl-1-phosphonate (CPP) (105% ± 5.7, n=14), when these antagonists were applied with currents which blocked responses to kainate and AMPA, or responses to NMA, respectively. The broad-spectrum antagonist kynurenate could antagonise responses to NMA, kainate and AMPA, whilst leaving responses to t-ACPD relatively unaffected (89% ± 8.5, n=16). In contrast, D-amino-4-phosphono-butyrate (AP4) was found to reduce responses to all agonists to a similar extent (*t*-ACPD 58% ± 12.7; AMPA 32% ± 6.7; NMA 37% ± 9.1 of control responses, n=9). Both L-AP4 (n=16) and L-amino-3-phosphono-propionate (L-AP3) (n=12) could have similar actions, but also had other, excitatory, actions on some neurones. These results suggest that t-ACPD may excite thalamic neurones via a receptor other than the NMDA receptor or the CNQX-sensitive-non-NMDA receptor, and it is possible that this is the metabotropic excitatory amino acid receptor. The non-selective actions of AP4 and AP3 would appear to limit their usefulness as antagonists of *t*-ACPD.

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380P PHARMACOLOGICAL SEPARATION BETWEEN AMPA AND KAINATE RESPONSES OF RAT CORTICAL SLICES BY A 2,3-BENZODIAZEPINE, METHOHEXITONE AND NICKEL

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Earlier investigation of α-amino-3-hydroxy-4-isoxazolepropionate (AMPA) and kainate pharmacology on cortical wedges using 6-nitro-7-sulphamoylbenzo[f]-quinoxalinedione (NBQX) was consistent with separate receptor subtypes for the depolarising effects of these two glutamate analogues (Lodge et al., 1991). We have continued this investigation with 1-(amino-phenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine, (GYKI 52466) which is an AMPA antagonist on rat hippocampal neurones (Tarnawa et al., 1989), with methohexitone recently shown to reduce an NBQX-resistant component of the kainate dose-response curve in neonatal rat spinal cord in vitro (Zeman & Lodge, 1991) and with nickel which we found showed some selectivity toward kainate on cortical slices (Palmer & Lodge, 1991). Using 500um wedge-shaped slices of rat neocortex in a grease-seal two compartment bath (Lodge et al., 1991), we have constructed dose-response curves to AMPA and kainate in the absence and presence of increasing concentrations of the above substances.

GYKI 52466 reduced responses to AMPA to a greater extent than those to kainate. Thus in the presence of 31.6μM, the dose ratio for AMPA was 11.3 and that for kainate was 2.6. Methohexitone and nickel had the reverse selectivity. Methohexitone, at a concentration of 31.6 μM, produced a dose ratio for AMPA of 1.2 and for kainate of 2.8. The dose ratios in the presence of 1mM nickel were 3.0 for AMPA and 7.5 for kainate. The effects of both these antagonists were reversible.

Although the prime aim of this study was to investigate AMPA and kainate pharmacology, it should be noted that responses to N-methyl-D-aspartate were less sensitive to GYKI 52466 than those to kainate, were equisensitive to methohexitone with those to AMPA and were more sensitive to nickel than were those to kainate.

The present results are further evidence that responses to kainate and AMPA are mediated by pharmacologically distinct receptors and are consistent with the hypothesis that part of the kainate depolarisation is due to an increase in calcium conductance.

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381P QUINOXALINEDIONES AND 2,3-BENZODIAZEPINES DO NOT DISCRIMINATE BETWEEN AMPA AND KAINATE RESPONSES ON SPINAL NEURONES IN HALOTHANE ANAESTHETISED RATS

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The recent study showing that responses to kainate on spinal neurones in vitro are selectively reduced by barbiturates (see Zeman and Lodge, this meeting) questions the validity of results from our earlier in vivo experiments which showed that quinoxalinediones did not distinguish between responses to kainate and α-amino-3-hydroxy-4-isoxazolepropionate (AMPA) on spinal neurones in pentobarbitone anaesthetised rats (Lodge et al., 1991). We have therefore re-examined the actions of 6-nitro-7-suphamoylbenzo[f]quinoxalinedione (NBQX; Sheardown et al., 1990) and the new AMPA antagonist, 1-(amino-phenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466; Tarnawa et al., 1989) on the increase in action potential frequency of single spinal neurones induced by electrophoretically adminstered N-methyl-D-aspartate (NMDA), AMPA and kainate in rats anaesthetised with 0.5-1.5% halothane in 66% N_2O in O_2.

Electrophoretic ejection of NBQX (5-20 nA; 1 mM in 200 mM NaCl; pH 8.1), tested on 24 cells, reduced responses to AMPA, kainate and NMDA by 71 ± 3% (mean ± S.E.), 62 ± 5% and 2 ± 2% respectively with good recovery in 5-15 min after stopping NBQX ejection. This compares with 68 ± 8%, 66 ± 4% and 7 ± 3% respectively for NBQX on cells in pentobarbitone-anaesthetised rats. Furthermore, intravenous injection of methohexitone 5 mg/kg or phenobarbitone 10-20 mg/kg had no selective effect on responses to these amino acids. Methohexitone (20-75nA; 25 mM in 175 mM NaCl; pH 9.7), tested on 24 spinal neurones in halothane-anaesthetised rats produced non-selective reduction of responses to AMPA, kainate and NMDA by 25 ± 8%, 20 ± 7% and 23 ± 7% respectively. Ejection of GYKI 52466 (15-100nA; 4mM in 200mM NaCl; pH 4.2) reduced responses to AMPA, kainate and NMDA by 43 ± 5 %, 58 ± 5 % and 17 ± 3 % respectively in halothane anaesthetised rats. This antagonistic effect of GYKI 52466 was rapid in onset and reversible within 10-20 min of stopping its ejection.

The present results suggest that barbiturates do not substantially or selectively affect the increase in firing rate of spinal neurones to kainate or AMPA. They are consistent with the notion that AMPA and kainate act largely via a pharmacologically identical subtype of glutamate receptor on these spinal neurones in vivo.

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382P A DIRECT HPLC ASSAY OF L-2-AMINO-4-PHOSPHONOBUTANOATE ACCUMULATION BY RAT HIPPOCAMPAL SLICES

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Previous studies on transport in brain of the ω-phosphonate analogue of L-glutamate, L-2-amino-4-phosphonobutanoate (L-AP4), have commonly employed the radiolabelled racemate (*e.g.* Bridges *et al.*, 1986). We now measure L-AP4 accumulation directly.

After a 15 min equilibration period, transverse hippocampal slices (400 μm) from 8 week-old rats were superfused and incubated for 4 min in an oxygenated Krebs'-bicarbonate buffer (KBB; 25°C) which contained L-AP4. Incubation was terminated by addition of ice-cold KBB and the slices were superfused in this medium until L-AP4 could no longer be detected in it (< 3 nM). After slice homogenisation, centrifugation, supernatant layer filtration and protein removal, the resulting filtrate was assayed for its L-AP4 content using reversed-phase HPLC with fluorescence detection (Harrison & Kilpatrick, 1991).

Table 1 Influence of low temperature and transport inhibitors on accumulation of L-AP4 by rat hippocampal slices

	EXTERNAL CONCENTRATION OF L-AP4 *200 μM*	*646 μM*
Control (25°C)	44.6 ± 8.6 (8)	158.8 ± 26.4 (6)
Ice-cold (2°C)	16.0 ± 3.4 (4) *	16.8 ± 2.3 (4) **
+ 100 μM Ouabain	16.9 ± 5.6 (5) *	31.5 ± 8.9 (3) *
+ 1 mM TßHLA	36.7 ± 8.8 (3)	142.7 ± 32.8 (3)
+ 1 mM L-*trans*-PDC	48.4 ± 10.4 (3)	149.0 ± 35.3 (3)
+ 1 mM L-HCA	22.4 ± 2.5 (4)	40.4 ± 11.8 (4) **
+ 1 mM SITS	20.2 ± 3.5 (4)	25.7 ± 5.7 (4) **

Each value is the mean ± s.e.mean L-AP4 content in *pmol/slice/4 min*. Except for ouabain (15 min before), each test agent was added simultaneously with L-AP4. The number of determinations is shown in parentheses. Using Student's *t*-test, the following indicators show values that were significantly different from control: * $P<0.05$, ** $P<0.005$

Accumulation of L-AP4 was markedly temperature-sensitive (Table 1), suggesting an active L-AP4 transport mechanism in brain. Although this sensitivity could be due to reduced extracellular receptor binding, the effect of 100 μM ouabain infers that at least part of the accumulation is due to active sequestration. Radiolabelled studies indicate that AP4 is principally transported via a Ca^{2+}/Cl^--dependent carrier (Bridges *et al.*, 1986). Indeed, inclusion of either *threo*-ß-hydroxy-L-aspartate (TßHLA) or L-*trans*-pyrrolidine-2,4-dicarboxylate (L-*trans*-PDC), inhibitors solely of Na^+-dependent excitatory amino acid transport, had little effect on L-AP4 accumulation. However, both L-homocysteate (L-HCA; a substrate for the Cl^--dependent site) and 4-acetamido-4'-isothiocyanato-stilbene-2,2'-disulphonate (SITS; a known inhibitor of this carrier), readily offset control accumulation of L-AP4.

Using this new assay technique, these preliminary results appear to confirm that L-AP4 transport in brain is predominantly via a site displaying a similar pharmacology to that of the known Cl^--dependent site. Further ionic dependence studies are in progress.

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383P EFFECT OF QX314 ON ACTION POTENTIALS IN HIPPOCAMPAL CA1 CELLS USING CURRENT-CLAMP AND VOLTAGE-CLAMP TECHNIQUES

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Hippocampal CA1 pyramidal cells in vitro can be excited via stimulation of afferent fibres to the proximal and distal apical dendrites (Andersen et al., 1987). The local anaesthetic QX314 (20-35mM) was injected intracellularly using standard current-clamp and whole-cell voltage-clamp techniques. The proximally and distally evoked synaptic responses were monitored over time. The current-clamp data showed the proximally evoked action potential (AP) to be blocked after 57±10 minutes (n=5), whereas the distally evoked AP was present up to 88±14 minutes (n=5). In contrast, QX314 introduced into cells via whole-cell electrodes caused an almost instantaneous block of all voltage-activated sodium currents. The excitatory postsynaptic potentials and currents (EPSP's and EPSC's) were still present after QX314 blockade. Current-voltage relationships show that the remaining inward current is carried by calcium ions.

The data suggests that there is more than one triggering site for AP's in pyramidal cells and that these sites are spatially separate. The proximally evoked AP may be triggered at either the soma or the initial segment, whereas the distally evoked somatic AP may be triggered by a dendritic spike which appears as a prepotential at the soma. In agreement with previous observations, whole-cell voltage-clamp data shows that QX314 blocks both the voltage-dependent inactivating as well as the non-inactivating sodium conductances. The presence of these remote spike trigger mechanisms may have implications for the action of excitatory neurotransmitters on these pyramidal cells.

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384P THE ROLE OF GLYCINE IN MODULATING THE N-METHYL-D-ASPARTATE (NMDA) COMPONENT OF THE EPILEPTIFORM BURST IN THE KAINIC ACID (KA) LESIONED HIPPOCAMPUS

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In previous electrophysiological studies of synaptic activity in the unilaterally KA lesioned rat hippocampus, we have demonstrated graded epileptiform bursting activity that is mediated by non-NMDA and NMDA receptors (Simpson & Wheal, 1990; Simpson *et al.*, 1991). The purpose of the present study was to determine the role of glycine in modulating the NMDA receptor component of the epileptiform burst in this model. Both intra- and extracellular recordings were made from the CA1 pyramidal cell layer in response to suprathreshold stimulation in the stratum radiatum.

6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) (2-5 μM) significantly blocked part of the epileptiform burst response. The effect of 7-Cl-kynurenate (7-Cl-KYN) on the CNQX insensitive component of the burst was examined. A concentration of 20 μM 7-Cl-KYN was initially chosen, however in only 1/4 extracellular and 2/5 intracellular experiments was this effective in blocking the CNQX insensitive component. In the remaining studies D-2-amino-5-phosphonovalerate (D-APV) (10-20 μM) was required to block the residual response. It was therefore necessary to determine why the 7-Cl-KYN was relatively ineffective in this *in vitro* preparation. The most obvious explanation was that 20 μM 7-Cl-KYN was insufficient to compete with a high concentration of glycine in the slice. In 6 extracellular and 3 intracellular protocols a combination of 5 μM CNQX and 40 μM 7-Cl-KYN successfully blocked the evoked burst response and underlying EPSP. The subsequent application of the glycine analogue, D-serine (200 μM), reversed some of this block (6/6 slices; 2/2 cells). To confirm that glycine levels in this slice preparation were sufficient to saturate the glycine site of the NMDA receptor channel complex, 200 μM D-serine was applied to the extracellular burst response. D-serine had no significant effect on the burst in 4/4 experiments. In conclusion, the NMDA receptor mediated component of the epileptiform burst depends upon activation of the glycine site. However, the physiological role for this site remains unclear.

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385P A COMPARISON OF THE ANTAGONIST SELECTIVITY OF NBQX AGAINST FIVE NON-NMDA RECEPTOR AGONISTS

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We have investigated the potency of 6-nitro-7-sulphamobenzo(f)quinoxaline -2,3-dione (NBQX) as an antagonist of depolarization of motoneurones induced by the non-NMDA receptor agonists acromelic acid A (0.3 - 1 μM), Domoic acid (0.1 - 0.3 μM), kainate (0.25 - 2 μM), (S)-α-amino-5-methyl-3-hydroxyisoxalole propionic acid (AMPA) (0.25 - 0.8 μM) and quisqualate (0.4 - 4 μM). Motoneuronal responses were recorded from ventral roots of hemisected spinal cord preparations, prepared from rats of three to five days post partum age, and bathed in medium containing 2 mM $CaCl_2$, no added magnesium and 0.1 μM tetrodotoxin. Control concentration-effect plots were obtained first and dose-ratios for antagonism were obtained following application of NBQX (10 μM) for at least 40 min. Mean $_pA_2$ values ± s.e.mean (number of preparations) obtained with each agonist are presented below.

Domoic	Kainic	Quisqualic	AMPA	Acromelic A
5.29 ±0.05(3)	5.72 ±0.07(3)	6.12 ±0.08(3)	6.80 ±0.09(4)	6.76 (2)

NBQX produced a parallel rightward displacement in most of these experiments. However, in one of the tests with kainate (43% reduction) one with acromelate A (84% reduction) and all three with quisqualate (mean reduction 46% ±6% s.e.mean) there was a marked reduction of the slope of the concentration-effect plot. In these cases the $_pA_2$ values were calculated at the mid-point of the control concentration range. These data conform with the reported AMPA receptor-selective profile of NBQX (Honoré et al, 1990). Thus domoate appears to be the least AMPA receptor-selective ligand of this series whereas the selectivity of acromelate A appears to be similar to that of AMPA itself in conformity with previous observations Watkins et al (1990). An interaction of NBQX with the metabotropic component of the response may possibly explain the flattening of the quisqualate response plots produced by NBQX.

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386P INVOLVEMENT OF NMDA AND NON-NMDA RECEPTORS IN MONOSYNAPTIC EXCITATORY TRANSMISSION IN THE CAT RED NUCLEUS *IN VIVO*

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The magnocellular neurones of the red nucleus (mRN) receive two distinct monosynaptic excitatory afferents, one from the contralateral interpositus nucleus (IPN) and one from the ipsilateral sensorimotor cortex (SMC). Previous studies have provided evidence that the transmitters in these pathways are excitatory amino acids (Nieoullon *et al.*,1984; Davies *et al.*, 1986). The aim of the present study was to establish the involvement of NMDA and non-NMDA receptors in these synaptic responses using selective antagonists of the excitatory amino acid receptors.

Cats were anaesthetised with α-chloralose (50 mg/kg i.v.) and extracellular recordings were obtained from the mRN cells using the central barrel (4M NaCl) of a multibarreled micro-electrode; the outer barrels containing the drug solutions for iontophoretic application. Excitatory responses were obtained by electrical stimulation of the contralateral IPN and the ipsilateral SMC (<200μA). Results were obtained from a total of 33 monosynaptically activated mRN neurones. The selective excitatory amino acid antagonists 2-amino-5-phosphonopentanoate (AP-5) and 6-nitro-7-sulphamobenzo[*f*]quinoxaline-2,3-dione (NBQX) were tested on synaptic responses and responses to NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA). AP-5 depressed or abolished the responses to NMDA while having no significant effect on AMPA responses. Similarly, NBQX depressed or abolished the effects to AMPA but not those to NMDA. The influence of these NMDA and non-NMDA receptor selective currents of AP-5 and NBQX respectively, were examined on monosynaptic excitation evoked by IPN and SMC stimulation. The results (mean ± SD) are summarised in Table 1.

Table 1. The effect of antagonists on the synaptic responses to IPN and SMC stimulation.

Drug	IPN stimulation			SMC stimulation		
	Mean ejection current (nA)	% Depression	No. depressed /No. tested	Mean ejection current (nA)	% Depression	No. depressed /No. tested
AP-5	68±33	0	0/10	30±22	51±33	21/25
NBQX	17±11	90±24	17/17	33±17	49±40	13/18

IPN-evoked responses were only sensitive to NBQX whereas SMC-evoked responses were depressed by both NBQX and AP-5. Indeed, in tests on 4 neurones, simultaneous ejection of both NBQX and AP-5 using currents of each that had no effect on their own depressed SMC-evoked responses by 70±21% (mean ± SD). Thus we conclude that both NMDA and non-NMDA responses mediate the synaptic excitation of the cortical pathway but that non-NMDA receptors mediate the response from the IPN.

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387P PRELIMINARY CHARACTERISATION OF THE MOLECULAR PROPERTIES OF NATIVE NON-NMDA RECEPTORS FROM RAT BRAIN

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Molecular cloning by functional expression has identified several cDNAs which encode glutamate receptors of the non-NMDA subclass e.g. GluR1-6(Egebjerg et al., 1991). In situ hybridisation studies, together with the co-expression of the different CDNAS, suggest that the natural receptors are hetero-oligomeric although the polypeptide compositions of these are not yet established. To approach the delineation of the subunit compositions of the in vivo receptors, we are attempting the characterisation of their molecular properties using biochemical and immunological methods.

High affinity [^{3}H] α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) binding sites in membranes from rat whole brain minus cerebellum were shown to display a pharmacological profile AMPA>CNQX≥glutamate>kainate with IC_{50} values respectively of 15 nM, 600 nM, 860 nM, 17500 nM. These were determined at 10 nM [^{3}H] AMPA and in the presence of 0.2 M potassium thiocyanate. The high-affinity AMPA binding sites were solubilised using 1% (v/v) Triton X-100 in the presence of 0.5 M sodium phosphate and assayed in 0.2 M potassium thiocyanate essentially as described by Hunter et al., 1990. Solubilised high-affinity [^{3}H] AMPA binding sites (IC_{50} = 6 nM) were purified 4-fold by wheat-germ agglutinin Sepharose affinity chromatography.

A partial cDNA was isolated from a size-selected rat λgt 10 library cDNA. Nucleotide sequencing identified it as corresponding to GluR1. A fusion protein was generated from this clone using a PGEX expression vector. The fusion protein was glutathione transferase with a C-terminal region which corresponds to part of the putative intracellular loop of the GluR1 subunit with size ≈ 8000 daltons. Polyclonal antibodies were raised in rabbits and immunoblot experiments showed that this anti-serum reacted with two major species with M_r 105000 and M_r 51000 from rat brain membranes or lectin affinity-purified preparations. The M_r 105000 immunoreactive species is consistent with the predicted molecular weight of the mature GluR1 subunit. Studies are in progress to further characterise the properties of this potentially useful antiserum.

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388P SPONTANEOUS EPILEPTIFORM DISCHARGE INDUCED IN MAGNESIUM-FREE MEDIUM IS NOT MEDIATED EXCLUSIVELY BY NMDA RECEPTORS IN RAT ENTORHINAL CORTEX *IN VITRO*

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As part of our studies on the monoamine inhibition of epileptiform discharge in the entorhinal cortex (see, for example, Sizer & Roberts, 1991), we are trying to establish in vitro models of epilepsy mediated exclusively either by NMDA or non-NMDA receptors. It has been reported previously that perfusion of a cortical brain slice with magnesium-free medium allows the development of epileptiform discharge that is completely abolished by the NMDA antagonist D-APV (Thomson & West, 1986; Jones & Heinemann, 1988). This activity presumably arises due to the release of the NMDA channel from its voltage-dependent block by magnesium ions (Nowak et al., 1984). Consequently, we set out to verify the NMDA receptor-dependency of this discharge in our parasagittal slice preparation of rat entorhinal cortex.

Conventional intracellular recordings were made in layers II/III of the entorhinal cortex and epileptiform activity was induced by switching the perfusion medium to one devoid of magnesium. Spontaneous discharge generally began to develop within thirty minutes. No stimulus protocol was necessary.
Once this activity had become stable, the NMDA antagonist D-APV was added to the perfusate at concentrations between 20 and 100uM. In all cells tested, 100uM D-APV was unable to block completely the epileptiform activity, although the discharge was greatly attenuated. The residual activity was abolished by the non-NMDA antagonist CNQX (10uM).

These data would seem to suggest that the epileptiform activity that develops in magnesium-free solution is also able to recruit non-NMDA receptors. Interestingly, a similar phenomenon has recently been reported in rat amygdala (Gean, 1990). Differences between these and previous results may be due to the cortical region studied or the orientation of the slice preparation.

A.R.S. is a Glaxo Research Scholar

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389P 4-AMINOPYRIDINE-INDUCED [^{3}H]-NORADRENALINE RELEASE FROM RAT HIPPOCAMPUS IS INDEPENDENT OF NMDA RECEPTORS

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4-Aminopyridine (4-AP) and related compounds induce a calcium- and tetrodotoxin-sensitive release of [^{3}H]-noradrenaline (NA) from hippocampal slices (Huang *et al.*, 1989; Hu & Fredholm, 1991). Since 4-AP might act by releasing glutamate, we have compared the 4-AP induced release to that induced by glutamate and N-methyl-D-aspartic acid (NMDA).

Rat hippocampal slices were prelabelled with [^{3}H]-NA and continuously superfused with Krebs-Ringer bicarbonate buffer containing additions as indicated (Hu & Fredholm, 1991). Evoked release is calculated by subtracting basal release and is expressed in percent of total tissue content.

4-AP caused a concentration-dependent (10-300 μM) release of [^{3}H]-NA ranging from 2.5 to 25%, which was independent of Mg^{2+} in the medium. NMDA (10-200 μM) and glutamate (300-3000 μM) caused an equivalent release that was virtually eliminated in the presence of Mg^{2+}. D-amino-5-phosphonopentoate (D-AP5), (+)-5-methyl-10,11-dihydro-5H-dibenzo (a,d)cyclohepten-5,10-imine maleate (MK 801) and kynurenic acid antagonized NMDA-induced release. D-AP5 and MK 801 reduced glutamate-induced release, whereas kynurenic acid was ineffective. CNQX (6-cyano-7-nitroquinoxaline-2,3,dione; 10-100 μM) blocked glutamate and 4-AP induced release. Glycine (100 μM) *per se* increased [^{3}H]-NA release by 2.5% and markedly enhanced NMDA and 4-AP induced release. This effect was strychnine sensitive. GABA (100 μM) caused a small release of [^{3}H]-NA and enhanced 4-AP, but not NMDA induced release.

The present results show that 4-AP induced [^{3}H]-NA release is independent of NMDA receptors, but may involve activation of non-NMDA receptors. Furthermore, glycine can enhance [^{3}H]-NA release independently of NMDA receptors.

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390P ACTIONS OF SOME HOMO- AND ISO-WILLARDIINES IN THE SPINAL CORD OF THE NEWBORN RAT

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(S)-Willardiine and 5-substituted analogues of this substance are potent excitatory amino acid (EAA) agonists acting at α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (Jane *et al.*, 1991). We have extended our investigation of structure-activity relations of this group of substances to include longer chain analogues (homowillardiines) and analogues in which the amino acid side chain is attached to other positions in the uracil ring (isowillardiines).

The substances were tested on the hemisected isolated spinal cord preparation of the 1-5-day old rat. Recordings were made from a ventral root of the responses of motoneurones to excitatory amino acid agonists and/or to electrical stimulation of the corresponding dorsal root. In a nominally Mg^{2+}-free perfusion medium both (R,S)-homowillardiine (500 µM-1 mM) and (R)-5-fluorohomowillardiine (100 µM), administered for 5 min, caused an EAA antagonist-like depression of spontaneous activity and of the slower (polysynaptic) components of dorsal root evoked ventral root potentials (DR-VRPs) while having little or no effect on the fast (monosynaptic) component. At the same concentrations these substances also antagonized NMDA-induced depolarizations.

(S)-3-Isowillardiine and (R,S)-5-isowillardiine both showed agonist activity; equieffective concentration ratios relative to (S)-willardiine = 1.0 were 5.9 and 40.2, respectively. Responses to (S)-3-isowillardiine (20-50 µM) were antagonized by 6-cyano-7-nitroquinoxalinedione (CNQX, 10 µM) but not by D-2-amino-5-phosphonopentanoate (D-AP5, 25 µM). Depolarizations produced by (R,S)-5-isowillardiine (100-200 µM) were insensitive to both D-AP5 (25 µM) and CNQX (10 µM), and decayed during continued administration of the substance suggestive of an action at desensitizing receptors. The (R,S)-6-isomer (1 mM) had a dual action, suggestive of agonist activity at AMPA receptors and antagonist activity at NMDA receptors, both of low potency.

These results extend previous observations of molecular features favouring activation of AMPA receptors and antagonism at NMDA receptors. The response produced by (R,S)-5-isowillardiine may reflect the activation of the metabotropic or a novel type of EAA receptor.

Jane,D.E.,Pook,P.C-K.,Sunter,D.C.,Udvarhelyi,P.M. & Watkins,J.C. (1991) This meeting.

391P *Trans*-ACPD-STIMULATED FORMATION OF INOSITOL PHOSPHATES IS SENSITIVE TO PROTEIN KINASE C ACTIVATION

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Trans-D,L-1-aminocyclopentane-1,3-dicarboxylic acid (trans-ACPD) is a selective agonist for the metabotropic excitatory amino acid (EAA) receptor (Palmer et al., 1989). We have investigated the ability of this ligand to influence phosphoinositide hydrolysis in guinea pig cerebrocortical slices (0.3 x 0.3 mm) and have considered the possible role of protein kinase C (PKC) in regulating functioning of the metabotropic receptor. In the presence of Li^+, trans-ACPD stimulated inositol monophosphate formation in a dose-dependent manner, with an EC_{50} of 48 ± 6μM and eliciting a maximal response of approximately 400% of basal levels.

Preincubation of slices with the PKC-activating phorbol ester, phorbol 12,13-dibutyrate (PDBu) (1 and 10 μM) reduced the trans-ACPD response to 40% above basal. NaF (10 mM), which will indirectly stimulate the hydrolysis of phosphoinositides by activation of the G-protein, was found to produce a 3 fold increase in inositol phosphate formation; this was insensitive to PKC activation by PDBu.

In order to further investigate the site of protein phosphorylation, G-protein α subunits, which have been proposed as substrates for PKC (Sagi-Eisenberg, 1989), were isolated by immunoprecipitation. The endogenous pool of ATP was labelled with [^{32}P]-phosphate (100μCi) and, following PDBu treatment, the subunits were isolated and their phosphorylation state determined by autoradiography. Our results failed to provide any evidence consistent with this proposal and we detected no apparent phosphorylation of the G-protein α subunits.

The results from this study show that the site of PKC action is neither at, nor downstream of the G-protein, suggesting that it is the receptor itself that becomes phosphorylated. This would agree with the identification of phosphorylation consensus sequences in the recently cloned receptor (Masu et al., 1991)

P.G. Jones is a Merck Student.

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392P EFFECT OF α_2-ADRENOCEPTOR ANTAGONISTS ON URINE OUTPUT IN NORMALLY-HYDRATED AND WATER-LOADED RATS

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The diuretic effects of α_2-adrenoceptor agonists in rats have been well-documented (Gellai, 1990). However, relatively few workers have examined the effects of α_2-antagonists on urine output. Thus we have measured the amount of urine produced by normally-hydrated and water-loaded (25 ml kg^{-1} p.o.) rats after treatment with idazoxan, RX811059 (Doxey et al., 1985) and the peripherally-acting α_2-antagonist L-659,066 (Clineschmidt et al., 1988). Male Wistar rats (350-450g) were injected i.p. and placed in metabowls (without food and water). Mean cumulative urine outputs (ml kg^{-1}; n=6-8) were measured at 2 and 6h and compared using ANOVA and Dunnett's test.

Table 1 Effect of α_2-antagonists on urine output in normally-hydrated (NH) and water-loaded (WL) rats

IDX mg kg^{-1}	NH	WL	RX mg kg^{-1}	NH	WL	L mg kg^{-1}	NH	WL
Vehicle	1.2±0.5	23.4±1.5	Vehicle	1.0±0.4	24.6±1.2	Vehicle	1.5±0.8	21.4±1.8
1	4.9±0.7*	25.5±2.2	0.3	1.4±0.8	21.3±1.7	1	2.9±1.4	18.2±1.0
3	3.8±0.4*	24.5±1.2	1	1.8±0.9	13.8±2.6*	3	1.4±0.7	14.4±1.9*
10	3.5±1.1	16.5±2.7*	3	2.2±1.3	13.8±2.5*	10	1.9±1.0	1.0±0.5*

Results represent mean urine outputs ± s.e.mean (ml kg^{-1}) in the 2h following drug administration; *P<0.05.

Idazoxan (1, 3 mg kg^{-1}) produced diuresis in normally-hydrated rats in the 2h following injection. However, RX811059 (0.3-3 mg kg^{-1}) and L-659,066 (1-10 mg kg^{-1}) had no effect on urine output. Idazoxan (10 mg kg^{-1}), RX811059 (1, 3 mg kg^{-1}) and L-659,066 (3, 10 mg kg^{-1}) decreased urine output in water-loaded animals. Cumulative 6h urine outputs of animals treated with RX811059 and L-659,066 were similar to controls. On the other hand, urine outputs of animals treated with idazoxan (10 mg kg^{-1}) were significantly higher than controls at 6h in both normally-hydrated (12.7±1.2 versus 6.1±1.4) and water-loaded (40.0±2.7 versus 28.6±1.3) rats. The antidiuretic effects of the selective α_2-antagonists RX811059 and L-659,066 suggest that α_2-adrenoceptors in the periphery may play a physiological role in the control of water balance. These findings confirm an earlier report using yohimbine (Gellai, 1990). The paradoxical diuretic effects of idazoxan, contrast with the effects of other α_2-antagonists, and therefore may be attributed to a property of this compound unrelated to α_2-adrenoceptor blockade.

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393P THE INFLUENCE OF PERFUSION PRESSURE AND ANGIOTENSIN II ON THE RENAL EXCRETORY RESPONSES TO ATRIAL NATRIURETIC PEPTIDES

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Atrial natriuretic peptide (ANP) has a potent action on the kidney to increase water and sodium excretion . The degree of natriuresis induced by ANP is variable but this may be due to a number of modulatory factors. Thus, we have recently shown (Chamienia & Johns) angiotensin II to decrease the renal excretory response to the synthetic ANP atriopeptin III (APIII). Reduction of renal perfusion pressure also decreases the effectiveness of ANP on the kidney, but this manoeuvre is also known to stimulate the release of renin and hence the production of angiotensin II. The aim of this study was to examine the effect of reduced renal perfusion pressure on the natriuretic response to APIII before and following blockade of the renin-angiotensin system with a converting enzyme inhibitor in order to separate the contribution of these two factors.

Male Wistar rats, 330-380g, were anaesthetised with sodium pentobarbitone and cannulae placed in the carotid and femoral arteries and jugualr vein. A retroperitoneal approach was used to allow cannulation of right and left ureters, to expose and allow fitting of an flow probe to the left renal artery and to place a thread around the aorta between the two renal arteries to permit manipulation of renal perfusion pressures. Saline and inulin was infused at 3ml h^{-1} and experiments were begun 2h later. Two sequences of five 15 min clearances were begun, 2 before and 2 after an experimental period at the start of which 1000ngkg^{-1} APIII was given i.v. The first set of clearances was obtained at normal perfusion pressure while the second was obtained when left renal pressure was kept at 80mmHg and that at the right allowed to rise to the higher prevailing level. Data was analysed by ANOVA and post-hoc tests for significant effects. In the first group of rats, blood pressure, at 121±2mmHg and left renal blood flow, at 5.1±0.4ml min^{-1}g^{-1}, was unchanged by the APIII challenge but it induced small (10%, $P<0.05$) increase in left glomerular filtration rate (GFR) at 1.2±0.03ml min^{-1}g^{-1} while urine flow (UV) at 8.6±1.3µlmin^{-1}g^{-1}, absolute sodium excretion (UNaV) at 1.89±0.19µmolmin^{-1}g^{-1} and fractional sodium excretion (FENa) at 1.17±0.12% increased by 85, 80 and 47% (all $P<0.01$), respectively. The right kidney exhibited similar patterns and magnitudes of responses to APIII. Reduction of left renal perfusion pressure to 80mmHg decreased renal blood flow and GFR slightly but caused much bigger falls in UV, UNaV and FENa of 70 to 90% and under these circumstances the second APIII challenge had no effect on any of the haemodynamic or excretory variables. By contrast, the right kidney, at a pressure of 130±1mmHg, had elevated levels of fluid excretion and the responses to APIII were larger (all $P<0.01$) than those obtained at normal pressures. Administration of captopril, 900µgkg^{-1}h^{-1} i.v., when perfusion pressure at the left kidney was reduced and that at the right was elevated, resulted in reversible increases in UV, UNaV and FENa in the left kidney, while the responses in the right kidney were significantly larger ($P<0.05$) than those obtained in the absence of captopril.

The results show that the magnitude of the excretory responses to ANP are directly related to the level of renal perfusion pressure. Blockade of the renin-angiotensin system enhances the responses to ANP at all levels of perfusion pressure. It is concluded that perfusion pressure is an important factor in determining the sensitivity of the kidney to ANP but that angiotensin II also exerts a small tonic inhibitory influence.

Chamienia, A.L. & Johns, E.J. (1991) Br. J. Pharmac. 103, 1893-1898.

394P THE DIFFERENTIAL CONTRACTILE EFFECTS OF NEUROMEDIN U-8 ANALOGUES ON THE ISOLATED RAT UTERUS

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The neuropeptide Neuromedin U-8 (NmU-8) was originally isolated from porcine spinal cord (Minamino et al., 1985). It is an amidated octapeptide: Tyr-Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH_2 and forms the C-terminus of the icosapentapeptide, Neuromedin U-25.

As a continuation of our initial studies to find the biological active domain within NmU-8 (Benito-Orfila et al., 1991), we have systematically replaced some of the original amino acids by leucine, a hydrophobic amino acid, and tested them on the rat uterus bioassay.

Porcine NmU-8 (custom synthesis; IAF Bio-Chem, Quebec, Canada), Leu^1-NmU-8, Leu^2-NmU-8, Leu^5-NmU-8 and Leu^6-NmU-8 (custom synthesis; Palliard Chemical Company Ltd, Twinstead, Sudbury, Suffolk, UK) were tested in the dose range 100 pM to 100 nM. All peptides, except Leu^6-NmU-8, showed a contractile effect in a concentration dependent manner on the rat uterus.

Female Wistar rats (150-200 g) were used. The animals were sacrified by asphyxiation in CO_2. The uterus was removed, mounted in an organ bath and maintained in Krebs solution at 30°C and pH 7.4, aerated with 95% O_2/5% CO_2. The tissues were equilibrated under a resting tension of 0.5 g and the isotonic muscular contractions were recorded as previously described (Domin et al., 1989).

The analogue Leu^1-NmU-8 (EC_{50}=0.60±0.03 nM) showed the same magnitude of response as that obtained with porcine NmU-8 (EC_{50}=0.61±0.04 nM). Leu^2-NmU-8 (EC_{50}=3.49±0.88 nM) and Leu^5-NmU-8 (EC_{50}=6.75±0.31 nM) showed a decrease in potency of 0.5 and 1 order of magnitude, respectively. Leu^6-NmU-8 was found to be inactive.

As shown by the results, proline is essential for bioactivity of NmU-8, since its replacement by leucine abolishes the contractile effect. The replacement of different amino acids by leucine induces a gradual loss of potency as follows: Leu^5-NmU-8 < Leu^2-NmU-8 < Leu^1-NmU-8 = porcine NmU-8.

This study indicates the importance of the different amino acids on the primary structure of Neuromedin U.

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395P PAF IS SYNTHESISED IN HUMAN COLON MUCOSA BY THE CDP CHOLINE:1-0-ALKYL-2-ACETYL-SN-GLYCEROL CHOLINE PHOSPHOTRANSFERASE (*DE NOVO*) PATHWAY

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The pro-inflammatory phospholipid platelet-activating factor (PAF) has been shown to be produced by the human colon mucosa in larger amounts in ulcerative colitis and Crohn's disease than in normal mucosa (Eliakim *et al.*, 1988; Kald *et al.*, 1990). PAF synthesis can occur via two major pathways; the de novo pathway, postulated to be responsible for maintaining basal levels of PAF, and the remodelling pathway which increases PAF production during inflammation. The de novo pathway is catalysed by a dithiothreitol insensitive CDP choline:1-O-alkyl-2-acetyl-sn-glycerol choline phosphotransferase which incorporates CDP choline into 1-O-alkyl-2-acetyl-sn-glycerol to form PAF. We studied this pathway in 1000g supernatants of homogenates of normal human colon mucosa prepared by centrifuging for 15 mins (4°C). Aliquots (≈600μg protein) were incubated with cytidine 5'-diphospho[methyl-^{14}C] choline ([^{14}C]CDP choline) 100nmoles of 1mCi/mmol in 25μl of TRIS-HCl buffer plus the substrate hexadecyl acetyl-sn-glycerol in a final concentration of 0.2mM at 37°C for up to 90 min in 0.1M TRIS-HCl buffer (pH 8) containing 1mg/ml BSA, 0.5mM EGTA, 10mM $MgCl_2$ and 10mM dithiothreitol. Final incubation volume was 1ml. After extracting with 1ml chloroform:methanol (2:1) the radioactive products were separated by silica gel TLC of the organic phase in chloroform:methanol:acetic acid:water (50:25:8:4).

Table 1 Radioactive products formed on incubation of [^{14}C]CDP choline, alkyl acetyl glycerol and human colon mucosa

Time (min)	Origin	Lyso PAF	PAF	Alkyl Acyl Phospholipid
10	45.4 ± 21.3	5.82 ± 5.50	48.8 ± 17.9	0
30	30.5 ± 16.8	17.7 ± 7.87	51.1 ± 10.8	0.65 ± 0.98
60	31.8 ± 20.1	31.1 ± 15.4	35.6 ± 12.1	1.51 ± 1.45
90	30.9 ± 1.82	53.8 ± 1.53	13.8 ± 3.16	1.50 ± 0.15

Values are means of data from 2 to 5 patients at each time point, mean ± S.D. Each incubation was replicated 2 to 4 times.
The table shows the products co-chromatographing with authentic standards as a % of the total radioactivity on the TLC plate.

PAF was rapidly formed; after 30 min incubation it decreased and one of its major catabolites lyso PAF increased. A small peak corresponding to a phosphatidyl choline dioleoyl standard was seen. In three experiments, the zone on the TLC plates co-chromatographing with the PAF standard was extracted. Activity in this zone eluted with authentic PAF on an HPLC column. Biological activity was also confirmed in this zone by aggregation studies. An at present unknown peak was detected at the origin in each experiment. In the absence of added hexadecyl acetyl-sn-glycerol no PAF was formed in a 60 min incubation (n=3). 54.0 ± 18.9% of PAF formed was metabolised in 60 minute incubation (n=5 ± S.D.). These data show that the de novo pathway of PAF synthesis is present in human colon mucosa.

We acknowledge the help of R. Jewell for the HPLC.
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396P INHIBITION OF NICOTINE-INDUCED RELAXATION OF THE BOVINE RETRACTOR PENIS MUSCLE BY NITRIC OXIDE SYNTHASE INHIBITORS

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It has been shown that in the bovine retractor penis muscle (BRP) the relaxation elicited by transmural stimulation of the NANC inhibitory nerves is inhibited by L-N^G-nitro arginine (L-NOARG) (Martin *et al.*, 1991), whereas it is not inhibited by L-N^G-monomethyl arginine (L-NMMA) (Gillespie & Xiaorong, 1989). It has further been reported that the BRP is relaxed by low concentrations of nicotine, acetylcholine (ACh) and DMPP, the principal point of attack probably being the cell bodies of the NANC inhibitory nerves (Klinge *et al.*, 1988). The purpose of this work was to investigate whether the nicotinic relaxation of the BRP is inhibited by L-NOARG, L-NMMA or L-NAME (N-nitro-L-arginine methyl ester).

Strips of the BRP were mounted for isometric tension recording in 20 ml organ baths containing Tyrode solution at 35°C aerated with 95% O_2 and 5% CO_2. Other details as well as the way of calculation of the IC_{50} values for the inhibition of the nicotine-induced relaxation were the same as those described for the assessment of ganglion-blocking activity (Alaranta *et al.*, 1990).

The IC_{50} value for the inhibitory effect of L-NOARG on the relaxation induced by 30 μM nicotine was about 2.4 μM. The effect of L-NAME was about 5 and that of L-NMMA more than 100 times weaker. Also the relaxations induced by ACh (100 μM) and DMPP (20 μM) were totally blocked by L-NOARG. A 100-fold excess of L-arginine reversed the effect of L-NOARG.

The results suggest that the L-arginine nitric oxide synthase pathway is involved in the relaxation of the BRP induced by low concentrations of these nicotinic agonists. They also support the view that the NANC inhibitory nerves activated by them are the same as those activated by transmural stimulation.

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397P THE GANGLION-BLOCKING ACTIVITY OF NEUROMUSCULAR BLOCKING DRUGS AS ASSESSED WITH THE ISOLATED BOVINE RETRACTOR PENIS MUSCLE

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It is commonly known that neuromuscular blocking agents vary with respect to their potencies in producing ganglionic blockade. Tubocurarine is known to be the most potent in this respect, while the relative potencies of the other agents are less exactly known. The aim of the present study was to assess with the same method the ganglion-blocking activity of eight neuromuscular blocking drugs that are in clinical use.

Inhibition of the nicotine-induced relaxation of the bovine retractor penis muscle (BRP) was chosen as the method. This is based on the fact that in the BRP there are sparsely scattered small ganglia and single nerve cell bodies (Alaranta *et al.*, 1989) which can be activated by various nicotinic agonists (Klinge *et al.*, 1988). It has also recently been reported that inhibition of the above-mentioned nicotine-induced relaxation is a suitable method for quantitative assessment of ganglion-blocking activity (Alaranta *et al.*, 1990).

The IC_{50} values, expressed as -log [M], for the neuromuscular blocking drugs studied in inhibiting the nicotine-induced relaxation of the BRP were in descending order as follows: tubocurarine 6.56, alcuronium 5.98, vecuronium 5.86, metocurine 5.79, pancuronium 5.34, atracurium 5.09, suxamethonium 3.26, and gallamine 3.07. As compared to the slope of the regression line, i.e. of the linear part of the concentration-effect curve, yielded by the ganglion-blocking activity of tubocurarine, the slopes of the regression lines yielded by the other neuromuscular blocking drugs were not significantly different except for that yielded by alcuronium.

The results confirm the idea that inhibition of nicotine-induced relaxation of the BRP is a useful method for the assessment of ganglion-blocking activity. They also make possible a more accurate comparison of this activity of the neuromuscular blocking drugs studied.

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398P EFFECT OF DOPEXAMINE HYDROCHLORIDE ON SYMPATHETIC NEUROEFFECTOR TRANSMISSION IN RABBIT ISOLATED BLOOD VESSELS

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Dopexamine hydrochloride is a sympathomimetic amine which is currently being evaluated for the treatment of several cardiovascular illnesses including congestive heart failure (Brown *et al.*, 1985; Foulds, 1988). Dopexamine is an agonist at peripheral β_2-adrenoceptors and dopamine receptors (DA_1 and DA_2). It is also a potent inhibitor of uptake-1 in postganglionic sympathetic neurones (Mitchell *et al.*, 1987; Nedergaard, 1989). In the present study, I investigated the effects of dopexamine on sympathetic neuroeffector transmission in rabbit isolated blood vessels.

Dopexamine ($3x10^{-8}$-10^{-6}M), cocaine (10^{-6}-$3x10^{-5}$M), and desipramine ($3x10^{-8}$-10^{-7}M) enhanced the contractions of pulmonary artery evoked by electrical-field stimulation. Corticosterone ($4x10^{-5}$M), corticosterone ($4x10^{-5}$M) plus cocaine ($3x10^{-5}$M), but not cocaine ($3x10^{-5}$M), attenuated the enhancement seen with dopexamine. High concentrations of dopexamine (10^{-5}-$3x10^{-5}$M), cocaine (10^{-4}M), and desipramine (10^{-6}-10^{-5}M) decreased the stimulation-evoked contractions. Dopexamine (10^{-7}-$3x10^{-5}$M), but neither cocaine nor desipramine, caused an increase in resting tension. A single concentration (10^{-7} and 10^{-6}M) of either dopexamine or desipramine caused a biphasic response as regards the stimulation-evoked contractions of pulmonary artery: initial enhancement followed by inhibition. Dopexamine, but not desipramine, caused an increase in resting tension that waned with time. Cocaine (10^{-5}M) caused a rapidly developing steady-state enhancement of stimulation-evoked contractions. At $2x10^{-4}$M, cocaine caused a corresponding inhibition. The inhibitory effect of dopexamine (10^{-6}M) and desipramine ($3x10^{-6}$M) was almost irreversible and partially reversible, respectively by washing the preparations with drug-free salt solution. The reduction caused by cocaine ($2x10^{-4}$M) and by prazosin (10^{-9}M) was fully reversed. Dopexamine (10^{-7} and 10^{-6}M) enhanced the ^{3}H-overflow evoked by electrical-field stimulation of rabbit pulmonary artery preloaded with ^{3}H-noradrenaline. The enhancement was continuous with time.

In the presence of cocaine ($3x10^{-5}$M) plus corticosterone ($4x10^{-5}$M), dopexamine (10^{-7}-10^{-5}M) antagonized competitively the contractions of rabbit aorta evoked by either noradrenaline ($3x10^{-7}$-$3x10^{-4}$M) or phenylephrine (10^{-8}-$3x10^{-4}$M). The pA_2 values were 6.64 (noradrenaline) and 6.90 (phenylephrine). Dopexamine non-competitively antagonized the contractions evoked by dopamine ($3x10^{-7}$-$3x10^{-4}$M). Dopexamine (10^{-7}-10^{-5}M) did not antagonize the contractions of aorta evoked by histamine ($3x10^{-7}$-$6x10^{-5}$M) and by 5-hydroxytryptamine ($3x10^{-7}$-$3x10^{-4}$M). Dopexamine (10^{-7}-10^{-5}M) slightly enhanced the contractions of aorta evoked by potassium (10-55 mM). Dopexamine (10^{-5}M) also antagonized competitively the noradrenaline-evoked contractions of the pulmonary artery. These results suggest that dopexamine is an inhibitor of the neuronal uptake mechanism and an α_1-adrenoceptor antagonist. Furthermore, dopexamine has no affinity to histamine and 5-HT_2 receptors.

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399P INTERACTION OF THE (+) AND (–) ISOMERS OF CROMAKALIM ON PULMONARY CHOLINERGIC NERVES

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We have previously reported that in an vitro isolated guinea-pig tracheal tube preparation, cromakalim inhibits transmission in the pulmonary cholinergic nerves (Hall & Maclagan, 1988) due to an action at the ganglia and the final nerve terminals (Cooper and Maclagan, 1990). In the present experiments the actions of cromakalim and its constituent (-) enantiomer lemakalim BRL38227 and the (+) enantiomer BRL38226 were compared. Contractions of the isolated guinea-pig tracheal tube, measured as a change in intraluminal pressure, were elicited by either preganglionic vagal stimulation via a ring electrode (PGS) or by transmural stimulation in the presence of hexamethonium, 50 μM (TMS). Indomethacin was present in all experiments to abolish prostaglandin-induced tone. Stimulus parameters were 30 V, 30 Hz, 0·2 ms, 5 s duration, 40 s interval between trains.

Neither cromakalim nor BRL38227 appeared to have any postjunctional action on muscarinic receptors since they did not affect rise in tracheal tone to exogenous acetylcholine.

The (-) enantiomer of cromakalim, BRL38227 (0·01-26 μM) caused a 100% inhibition of both PGS and TMS with an IC50 of 0·24 μM and 0·06 μM respectively. Cromakalim (0·1-26 μM) however, produced a maximum of only 65% inhibition of PGS and 53% inhibition of TMS at 26 μM with ½ maximum inhibition at 3 μM (PGS) and 12 μM (TMS).

The (+) isomer of cromakalim, BRL38226, is considered to be relatively inactive. BRL38226 (0·1 to 26 μM) caused only a very slight inhibition of responses to TMS and PGS. However in the presence of the (+) isomer (26 μM) the inhibitory action of the (-) isomer was significantly reduced so that inhibition of PGS produced by BRL38227 was reduced from 90·46 ± 4·60 % to 33·7 ± 8·65 % at 1·6 μM and from 100 % to 51·46 ± 7·49 % at 26 μM. Similarly the inhibitory effect of BRL38227 on TMS was reduced from 93·25 ± 3·48 % to 40·23 ± 7·13 at 1·6 μM and from 100 % to 56·25 ± 4·71 at 26 μM. It is possible that BRL 38226 may be a partial agonist for the neuronal K^+ channels

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400P BRONCHOCONSTRICTION BY INHALED ADENOSINE IN GUINEA-PIGS PRETREATED WITH OVALBUMIN, PAF OR OZONE

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Although inhaled adenosine (ADO) has no effect in normal subjects, it induces bronchoconstriction in asthmatic patients (Cushley et al. 1983). In the present study we have compared the effects of inhaled ADO in normal guinea-pig and in three models known to be associated with airway hyperreactivity. These were 1) sensitization with ovalbumen (OA) at 14 (5mg ip) and 12 days (10mg ip) before use, 2) exposure to PAF-acether (0.01mg ml^{-1}) by aerosol for 60s and 3) exposure to an atmosphere of ozone (1.4ppm) for 1h.

The airway responses of conscious male Dunkin-Hartley guinea-pigs to a 1 min exposure of aerosolized ADO (1mg ml^{-1}) was measured by whole body plethysmography as the change in specific airway conductance (sGaw) (Griffiths-Johnson et al. 1988). In untreated animals ADO induced bronchodilatation, the peak increase in sGaw (34±8%, n=6) occurring at 15 min. In contrast, animals sensitized to OA responded to ADO with a bronchoconstriction, sGaw being reduced by a peak of 24±4% at 15 min (n=6).

Exposure to PAF caused a bronchoconstriction, the peak reduction of sGaw (25±5%) occurring at 5 min with return to baseline at 1h. At this point, the animals were exposed to ADO, which caused bronchoconstriction, sGaw being reduced by 7±4% at 15 min (n=6). This compared with the bronchodilatation (sGaw increased by 32±12%) seen in the same animals 1 week before.

Ozone caused bronchoconstriction, the reduction in sGaw at 30 min being 20.7±17%, with return to baseline after 1h. At 1h after the end of ozone exposure, ADO produced a powerful bronchoconstriction, sGaw decreasing by 39±6% at 20 min (n=6). This compared with the bronchodilator response to ADO (sGaw increased by 15±10% at 20 min) seen in the same animals 1 week before.

Thus in common with the situation in asthma, ADO was bronchodilator in normal guinea-pigs, but after induction of experimental bronchial hyperreactivity, ADO was bronchoconstrictor. These models will thus assist in the further evaluation of the mechanisms of ADO in asthma.

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401P K^+-RICH MEDIA AND THE RELAXANT ACTIONS OF BRONCHODILATOR DRUGS IN GUINEA-PIG TRACHEALIS

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In guinea-pig isolated trachealis, charybdotoxin (60-100nM) antagonises the relaxant effects of isoprenaline, salbutamol, aminophylline and theophylline without antagonising cromakalim, pinacidil or RP 49356 (Jones et al., 1990; Murray et al., 1991). Since charybdotoxin is a relatively selective inhibitor of large, Ca^{2+}-dependent K^+-channels (BK_{Ca}), this suggests that BK_{Ca} channel opening contributes to the relaxant actions of isoprenaline, salbutamol, aminophylline and theophylline. To determine the importance of BK_{Ca} channel opening in the relaxant action of isoprenaline, Jones et al. (1990) constructed log concentration-relaxation curves for isoprenaline against tracheal tone induced by carbachol (0.34μM). Following the addition of 80mM KCl to the bathing medium, the log concentration-relaxation curve for isoprenaline was virtually ablated. Jones et al. (1990) therefore suggested that the relaxant action of isoprenaline was very dependent on its ability to open BK_{Ca} channels and thereby to hyperpolarise the plasmalemma.

We have now measured the effects of K^+-rich media on the tracheal relaxant effects of isoprenaline, salbutamol, theophylline and cromakalim essentially as described by Jones et al. (1990). When K^+-enrichment (80mM) of the indomethacin (1.4μM) and carbachol (0.34μM) -containing medium was performed simply by addition of the appropriate amount of KCl, the log concentration-effect curves of each of the relaxants were virtually ablated. In contrast, when K^+-enrichment was performed with simultaneous reduction in NaCl to maintain osmolality, the concentration-effect curve of cromakalim was ablated. The curve for salbutamol was shifted (7 fold) to the right with marked (>50%) depression of the maximal response. The curve for isoprenaline was also rightward shifted (4 fold) and the maximal response was modestly (20%) reduced. The curve for theophylline was rightward shifted (2.4 fold) but no evidence for a reduction in maximal response was obtained. Similar results were obtained in a parallel series of experiments where concentration-relaxation curves for the bronchodilator drugs were initially constructed against the spontaneous tone of the tissue in an indomethacin- and carbachol-free medium.

We conclude that the carbachol-containing, K^+-rich medium used by Jones et al. (1990) does not descriminate between bronchodilator drugs whose actions are totally or partially dependent on K^+-channel opening. The present results also support our earlier conclusion (Allen et al., 1985) that K^+-channel opening (and subsequent inhibition of Ca^{2+} influx due to cellular hyperpolarisation) may contribute to, but is not crucial for, the tracheal relaxant action of isoprenaline.

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402P SALMETEROL INHIBITS THE RELAXING EFFECT OF FORMOTEROL ON GUINEA-PIG TRACHEAL SMOOTH MUSCLE: EVIDENCE FOR PARTIAL AGONISM

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Formoterol and salmeterol belong to a new generation of β_2-selective adrenoceptor agonists characterized by a long duration of action when inhaled. Formoterol is approximately fifteen times more potent than salmeterol in relaxing tracheal smooth muscle (Nials et al., 1990), but a direct comparison of the relative efficacies of the two compounds does not seem to have been published. We now show that in relation to formoterol, salmeterol behaves as a partial agonist.

Tracheal strip preparations from guinea-pig were precontracted with 1 μmol/l carbachol. With this concentration a near maximum contraction is produced. Formoterol was then added cumulatively with or without salmeterol added to the organ bath at various concentrations (from 0.1 to 10 μmol/l) 1 h beforehand. The time interval between the increments of formoterol was 10-15 min. In some experiments the nonselective agonist, isoprenaline was substituted for formoterol. Maximum relaxation was established by the final addition of 1 mmol/l theophylline. Calculation of pA_2-values was made using the Schild equation according to McKay (1978).

Under the present conditions salmeterol in concentrations up to 10 μmol/l caused only a partial relaxation (about 30 per cent) of the tracheal smooth muscle whereas the relaxation brought about by formoterol was near maximum with a $pD_2$of 8.56±0.05 (mean±SE; n=26). Moreover, salmeterol caused a concentration-dependent rightward shift of the concentration-response curve for formoterol. The pA_2-value for salmeterol calculated from this shift was 7.42±0.08 (mean±SE ; n=30). This value is compatible with the affinity constant for salmeterol determined on β_2-adrenoceptors in rat lung membranes (Brittain et al., 1988). The relaxation by isoprenaline was only slightly inhibited by salmeterol. This may indicate that salmeterol has a very low affinity for β_1-adrenoceptors. When β_2-adrenoceptors are blocked selectively, isoprenaline relaxes the guinea-pig tracheal smooth muscle via β_1-adrenoceptors (Johansson et al. 1984).

The present data clearly show that salmeterol is a partial β_2-adrenoceptor agonist in relation to formoterol. The clinical significance of this difference in efficacy remains to be elucidated.

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403P EFFECTS OF KHL-8425 ON THE AIRWAY CILIARY ACTIVITY AND ON RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

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KHL-8425, 3,7-dihydro-3-methyl-7-((5-methyl-1,2,4-oxadiazol-3-yl)methyl)purine-2,6-dione is an active metabolite of Chinoin-170, a C7-substituted theophylline derivative (Minker et al., 1990). KHL-8425 has a potent, long-lasting antitussive activity without adverse effects on the cardiovascular or central nervous systems (CNS; Korbonits et al., 1990).

We measured the ciliary beating frequency (CBF) photoelectrically from human sinus mucosal explants which were immersed in solutions of KHL-8425 (0, 0.1, 0.2, 0.5 mg/ml). CBF was measured before drug exposure and after 10, 20, 40, 60 and 90 min immersion periods. In vivo, CBF was measured from incised tracheas of anaesthetized Dunkin-Hartley guinea pigs. CBF was measured before and 8, 16, 24, 32, 40, 48, 56 and 64 min after intravenous injection of KHL-8425 (2 or 5 mg/kg; 1 ml/kg of vehicle to controls).

In human mucosa, CBF was 15.2 - 17.2 beats/sec in explants immersed in vehicle; KHL-8425 had no effect on CBF. In guinea pigs, CBF (baseline 15.8 beats/sec) decreased in control animals by 5.9% during the 64 min experiment. After 2 mg/kg of KHL-8425, CBF increased by 3.6 - 13.7 %, but the effect was not time dependent. After 5 mg/kg dose, no consistent changes were seen. The effect of KHL-8425 on various CNS receptors was studied using tritiated ligands and homogenates of Wistar rat brains. KHL-8425 showed no affinity for central- and peripheral type benzodiazepine receptors, GABA-A receptors, opioid μ-type and δ-type receptors, tryptamine receptors and 5-HT type 2 receptors producing IC50 values above 1000 μM.

Impairment of ciliary function may increase the susceptibility to bacterial infections. KHL-8425 lacks the ciliostatic action common to many antitussives, e.g. codeine and dextromethorphan (Karttunen et al., 1990; Karttunen et al., 1991). The lack of affinity for various CNS receptors suggests good tolerability without sedative or euphoria-inducing effects.

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404P NEDOCROMIL SODIUM MODULATES THE ABILITY OF RAT PERITONEAL MAST CELLS TO DEGRADE NEUROPEPTIDES

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The vasodilator neuropeptide calcitonin gene related peptide (CGRP) is degraded to non-vasodilator fragments in skin by proteases from activated mast cells (Brain and Williams 1988). Nedocromil sodium inhibits the activation of and mediator release from cells that include rat peritoneal mast cells (Wells et al., 1986). We have examined whether nedocromil sodium can modulate the ability of activated mast cells to degrade CGRP and the neuropeptide vasoactive intestinal peptide (VIP).

Mixed peritoneal cells were obtained from female Wistar rats (200-300g) and washed. Samples (1ml) containing 1-3.10^5 mast cells were incubated in 1 ml modified Tyrode for 15 min at 37^{0}C with 5 fmol ^{125}I-VIP or ^{125}I-CGRP and test agents as required. Incubations were terminated by centrifugation and removal of supernatant. The intact peptide was then precipitated by 10% trichloracetic acid and separated from peptide fragments by centrifugation. Samples were then counted for radioactivity (Paul et al., 1989).

Compound 48/80 (0.03-1 μg/ml) acted in a dose-dependent manner to stimulate mast cell-dependent break down of both CGRP and VIP. Compound 48/80 (0.1 μg/ml) caused the degradation of 2.08 $\pm$ 0.3 fmol ^{125}I-VIP (mean +s.e.mean, n=12). The effect of nedocromil sodium on the ability of mast cells to inhibit this degradation of VIP is shown in table 1.

Table 1. % inhibition of ^{125}I-VIP breakdown by nedocromil sodium.

Nedocromil sodium	% inhibition	n	p (ANOVA)
10^{-6}M	24.5 $\pm$ 9.2	12	ns
10^{-5}M	29.2 $\pm$ 8.7	12	<0.05
10^{-4}M	52.0 $\pm$ 11.6	12	<0.05
10^{-3}M	72.4 $\pm$ 20.7	4	-

In addition nedocromil sodium, at high doses, inhibited the degradation of ^{125}I-CGRP by Compound 48/80-activated mast cells; 41.6 $\pm$ 10.6 % inhibition of ^{125}I-CGRP degradation by nedocromil sodium (10^{-3}M), mean $\pm$ s.e. mean, n = 5.

These results suggest that nedocromil sodium inhibits mast cell-dependent break down of vasoactive peptides. This is probably due to inhibition of release of mast cell proteases by nedocromil sodium. This could contribute to the therapeutic effects of nedocromil sodium in the lung where, among other actions, VIP is known to possess bronchodilator activity and CGRP vasodilator effects.

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405P EFFECT OF R59022, AN INHIBITOR OF DIACYGLYCEROL KINASE, ON MEDIATOR RELEASE FROM HUMAN MAST CELLS AND BASOPHILS

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The crosslinking of IgE on the surface of human mast cells and basophils leads to the synthesis of lipid mediators and the release of histamine from the granules. Despite intense interest in signal transduction pathways relatively little is known about IgE-dependent mechanisms in human cells. We have previously shown that the crosslinking of IgE leads to an increase in membrane associated protein kinase C (PKC) and that agents which inhibit PKC ablate the subseqent release of histamine (Warner & MacGlashan, 1990). To test the hypothesis that alterations in PKC activity are directly related to the release of histamine we investigated the effect of R59022, a specific inhibitor of DAG kinase (de Chaffoy de Courcelles *et al*, 1985), on IgE-mediated release of histamine from human mast cells and basophils.

When basophils were pre-incubated with 1μM R59022 anti-IgE-induced histamine release increased from 19 ± 5% of total to 43±8% of total (n=7, $p<0.05$). The drug also increased f-met peptide induced histamine release from 18±4% of total to 38±17% of total (n=5, $p<0.05$). This increased histamine release was not due to cytotoxic effects of R59022 as the drug alone did not initiate histamine release nor did the drug potentiate degranulation caused by phorbol ester (n=4). The drug (10μM) increased histamine release significantly at all concentrations of anti-IgE though it was less effective at higher (>1% w/v) doses. As anticipated, we found a negative correlation ($r=0.77$, $p<0.05$) between control histamine release and the enhancement when the cells were pre-incubated with 10μM R59022. In contrast, the drug failed to increase anti-IgE induced histamine release from human lung mast cells. In the absence of drug, anti-IgE (1% w/v) induced the release of 29±11% of the total histamine, a value which was unchanged by the addition of up to 100μM R59022 (n=4).

In summary, R59022 increased the receptor mediated release of histamine from human basophils but failed to affect non-physiologic stimuli such as the phorbol ester. However, the drug did not affect the release of histamine from human lung mast cells highlighting the differences between human Fcε R1 bearing cells.

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406P THE EFFECT OF TYROSINE KINASE INHIBITORS ON MEDIATOR RELEASE FROM HUMAN BASOPHILS

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Recent investigations have focussed on the role of tyrosine kinases as second messengers in many cell systems. It has been shown that the crosslinking of IgE in rodent mast cells is associated with an increase in tyrosine phosphorylation and subsequent release of histamine (Kawakami *et al.*, 1991). To determine whether similar changes occur in the human mast cells and basophils we have examined the effects of two tyrosine kinase inhibitors on the release of histamine.

Basophils were preincubated with tyrosine kinase inhibitors (10 mins, 37°C) prior to the addition of either anti-IgE, f-met peptide or the phorbol ester, PMA. The results, expressed as % inhibition, are given below.

	Anti-IgE (0.1 % w/v)	F-met peptide (1μM)	PMA (10 ng/ml)
Genistein (10 μM)	47±12 (n=5, $p<0.05$)	15±12 (n=5, p=NS)	7±4 (n=5, p=NS)
Tyrphostin (10 μM)	26±9 (n=3, $p<0.05$)	3±3 (n=3, p=NS)	-1±4 (n=3, p=NS)

Neither of the tyrosine kinase inhibitors used were cytotoxic, since incubation of basophils with drug alone gave < 5% histamine release. Nor did the drugs affect the PMA-induced release of histamine suggesting that the inhibition was not due to effects on non-tyrosine kinases such as protein kinase C. Preliminary experiments (n=2) suggest that the drugs are less potent in human lung mast cells, indicating that there may be differences in the signal transduction pathways in these two cell types.

In summary, genistein (1-100μM) inhibits anti-IgE induced histamine release from human basophils and appears to show some selectivity for IgE-mediated release. It is less potent in the human lung mast cell. The second tyrosine kinase inhibitor, tyrphostin (1-100μM), is less potent than genistein but also shows some selectivity for anti-IgE induced histamine release.

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407P EFFECT OF SURFACE CONTACT ON HISTAMINE RELEASE FROM HUMAN MAST CELLS AND BASOPHILS

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Recent studies have shown that contact with a surface prior to the addition of the stimulus alters the ability of human neutrophils to release superoxide (Laurent *et al.*, 1991). The crosslinking of IgE has been shown to lead to the rapid upregulation of adherence receptors on the membrane of human basophils (Bochner *et al.*, 1988). To assess the relationship between adherence and cell responsiveness we have compared histamine release from human basophils in suspension or allowed to adhere to a range of different surfaces.

Basophils placed in contact with a siliconized plastic tissue culture plate, released significantly less histamine ($n=5$, $p<0.05$) than did cells maintained in suspension in a shaking waterbath. Peak histamine release was reduced from 48±17% of total to 26±4% of total. The difference was not due to a shift in the anti-IgE dose-response curve as normalized curves could be superimposed. The inhibition was independent of the nature of the surface because similar histamine release was obtained from basophils allowed to adhere to siliconized plastic wells, collagen coated plastic wells and confluent human endothelial cells. Nor did removal of up to 90% of the contaminating cells on Percoll density gradients affect the release of histamine suggesting that the effect was not due to the cell density. Preliminary experiments with human lung mast cells ($n=2$) suggest that, unlike the basophils, their responsiveness to different stimuli is not affected by proximity to a surface.

In summary, we have shown that human basophils in contact with a surface have reduced histamine release compared to cells maintained in suspension. The nature of the surface does not affect the degree of inhibition suggesting that it is mainly steric rather than requiring specific adherence receptors. In contrast, the responsiveness of human lung mast cells does not appear to be affected by the proximity of a surface.

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408P INHIBITION OF 15-LIPOXYGENASE BY ANTIDEPRESSANT DRUGS

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Barnes *et al* (1986) reported that amitriptyline had both bronchodilator and antidepressant properties which made it useful in treating a combination of asthma and depressive illness. Leukotrienes are potent bronchoconstrictors, and are known to be produced in asthma. The bronchodilator effect of amitriptyline may be brought about by inhibition of lipoxygenase. To investigate this possibility we have compared the inhibitory action of the known lipoxygenase inhibitor, nordihydroguaiaretic acid (NDGA) with the inhibitory effects of the antidepressant drugs, amitriptyline, imipramine, desipramine, mianserin and viloxazine.

Lipoxygenase activity was determined by measurement of the absorbance of the 1,3-diene product, 15-HPETE (Pretus *et al*, 1985). 15-HPETE, unlike the 1,4-diene structure of arachidonic acid, strongly absorbs at 240nm. The incubates contained 5.47μM arachidonic acid and 15μg soybean lipoxygenase in 0.1M MOPS buffer, pH7.4. Enzyme reactions were performed in the presence and absence of each of the anti-depressant drugs. Results represent the mean ± s.e. mean of 5 determinations.

Table 1 — Inhibition of 15-Lipoxygenase

	K_i	Nature of Inhibition
NDGA	1.20 ± 0.12 μM	Competitive
Amitriptyline	5.75 ± 0.17 μM	Competitive
Imipramine	55.0 ± 1.20 μM	Competitive
Desimipramine	13.5 ± 1.12 μM	Competitive
Mianserin	29.0 ± 0.92 μM	Uncompetitive
Viloxazine	No effect	

Most of the anti-depressant drugs tested (Table 1) inhibited 15-lipoxygenase with amitriptyline clearly the most potent. The three tricyclic antidepressants, amitriptyline, desimipramine and imipramine all produced a competitive inhibition of lipoxygenase. Viloxazine, a bicyclic antidepressant, had no effect on the enzyme whereas, mianserin, a tetracyclic compound, showed uncompetitive inhibition. The concentrations necessary to produce these effects are higher than those normally achieved in antidepressant therapy with these drugs (De Oliveira *et al* 1989)

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409P N-ACETYL CYSTEINE AMELIORATES HYPEROXIC LUNG INJURY IN THE PREMATURE GUINEA-PIG

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N-acetyl cysteine (NAC) is proposed to be a stimulator of glutathione (GSH) biosynthesis (Issels *et al.* 1988), and has been reported to increase circulating and bronchoalveolar lavage (BAL) concentrations of the tripeptide in animals and humans. In the present study the efficacy of NAC as a protective agent against hyperoxic lung injury was assessed in a recently developed guinea pig model of prematurity.

Timed pregnant guinea pig dams were delivered of their pups by Caesarian section on day 65 of gestation (term is 68 days). Pups were housed with surrogate mothers in normoxic (21% oxygen), or hyperoxic (95% oxygen) conditions for 72 hours. Pups were injected with 400 mg/kg body weight NAC i.p, or saline i.p, twice daily. Pups were sacrificed on the fourth day and BAL performed.

Preterm pups exposed to 95% O_2 showed elevated levels of GSH in both lung (1.62±0.11 95% O_2 vs 0.64±0.08 nmol/g 21% O_2, $P<0.05$) and liver (4.19±1.23 95% O_2 vs 1.95±0.43 nmol/g 21% O_2, $P<0.05$) compared to normoxic controls. There was no change in GSH concentrations of kidney, blood or BAL. Hyperoxic exposure was characterised by an increase in BAL fluid (BALF) protein content (0.66±0.07 95% O_2 vs 0.33±0.07 mg/ml 21% O_2, $P<0.05$). BALF protein is an index of oedema in pulmonary injury (Kelly *et al.* 1991).

NAC administration did not alter tissue, blood or BALF GSH content, either in normoxic or hyperoxic conditions. NAC did, however, reduce the BALF protein content in hyperoxia-exposed pups (0.32±0.05 NAC vs 0.66±0.07 mg/ml saline, $P<0.05$). The apparent amelioration of lung injury was offset by toxic effects of NAC treatment at this dose, as evidenced by increased mortality (Saline 21% O_2 25%, NAC 21% O_2 50%, Saline 95% O_2 42%, NAC 95% O_2 60%), and increased weight loss (16.7±3.0% birth weight NAC vs 4.5±2.73% saline, $P<0.05$).

The activities of the antioxidant enzymes catalase and glutathione peroxidase in the lung were unchanged by NAC treatment. The effect of NAC on lung injury cannot therefore be ascribed to the induction of these activities.

NAC appears to reduce the toxic effects of high oxygen concentrations on the lung, without altering GSH status. This may be a consequence of the reported antioxidant activity of NAC (Aruoma *et al.* 1989).

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410P ALINIDINE: AN IMIDAZOLINE WHICH ANTAGONISES THE EFFECTS OF CROMAKALIM IN ISOLATED BLOOD VESSELS

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The imidazoline derivative, alinidine is a potent bradycardic agent which inhibits pacemaker currents in cells of the rabbit sino-atrial node (Satoh & Hashimoto, 1986) and antagonises the vascular relaxant effects of the potassium channel opener cromakalim (McPherson & Angus, 1989). In the present study, the possibility that alinidine blocks potassium channels in vascular smooth muscle and thereby inhibits the effects of cromakalim was addressed.

The mechanical effects of alinidine were determined in the spontaneously active tissue of rat portal vein and against cromakalim-induced relaxation of 25mM KCl-induced contractions in rat aorta. The ability of alinidine to inhibit cromakalim-stimulated ^{42}K efflux from the rat aorta was also determined.

Rat portal veins, mounted under 1g resting tension showed regular phasic contractile activity. Alinidine (0.3-30μM) produced an increase in the integral of phasic contractions (maximum increase 232.4% ± 39.23 of basal level compared to 64.6% ± 10.39 in controls). This was accompanied by an increase in the amplitude and a reduction in the frequency of the contractions, compared to time-matched control tissues. At concentrations above 30μM, alinidine produced a marked increase in both resting tone and phasic mechanical activity of the portal veins followed by cessation of phasic contractions at the higher concentrations. These effects of alinidine were easily reversed on washout. Cromakalim (0.01-3μM) produced a concentration-dependent relaxation of rat aortic strips pre-contracted with 25mM KCl. Following pre-incubation of strips with alinidine (10 or 100μM) the relaxant curves to cromakalim were shifted to the right of time-matched control tissues. Alinidine did not affect resting tone in these tissues. In similar strips of rat aorta cromakalim (1μM) produced an approximate 3-fold increase in the ^{42}K efflux rate. Alinidine (100nM -100μM) had little effect on resting ^{42}K efflux from the aorta but produced a concentration-dependent inhibition of the cromakalim-stimulated ^{42}K efflux with full inhibition being obtained with 10μM alinidine.

This study has shown that in both venous (portal vein) and arterial (aorta) smooth muscles of the rat, alinidine exhibits potassium channel blocking properties. In addition alinidine inhibits both cromakalim-induced relaxations of and ^{42}K efflux from rat aorta. It is concluded that alinidine might prove to be a useful tool to characterise further the pharmacology of smooth muscle potassium channels.

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411P RELAXATION TO NITRIC OXIDE DOES NOT REQUIRE SMOOTH MUSCLE HYPERPOLARIZATION IN THE RABBIT ISOLATED BASILAR ARTERY

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Stimulation of muscarinic receptors in the rabbit isolated basilar artery, is followed by hyperpolarization and relaxation of the smooth muscle cells (Rand & Garland, 1991). These responses are qualitatively similar to the reported action of cholinomimetics in a number of other arteries (Bolton et al, 1984; McPherson & Angus, 1991). Recently, Tare et al (1990) have shown that nitric oxide, as well as inducing smooth muscle relaxation, can also hyperpolarize the muscle membrane. As the release of nitric oxide is thought, in most cases, to be responsible for the endothelium-dependent relaxation to acetylcholine and other agonists, this raises the possibility that the associated hyperpolarization is, at least in part, stimulated by nitric oxide.

Nitric oxide gas in concentrations up to 15μM (n=4), did not alter the smooth muscle membrane potential. If noradrenaline (10μM) was used to depolarize (from -59mV to -43mV) and contract the smooth muscle cells, the subsequent addition of nitric oxide gas again failed to induce membrane hyperpolarization, but did stimulate transient muscle relaxation. The threshold concentration for relaxation was 0.5μM, and the highest concentration tested, 15μM, stimulated over 80% relaxation (n=8). Sodium nitroprusside, which generates nitric oxide, produced very similar responses. If the smooth muscle cells were stimulated with noradrenaline, nitroprusside stimulated relaxation without a change in membrane potential in concentrations up to 100μM (91% relaxation; n=5). With higher concentrations, a slowly developing, reversible hyperpolarization of around 3mV with 100μM nitroprusside increasing to 19mV with 10mM was recorded (n=4). Similar responses occurred in the absence of noradrenaline. Both the smooth muscle hyperpolarization and relaxation to acetylcholine were reduced in the presence of the competitive substrate inhibitor for nitric oxide synthase, L-NMMA (10-100μM; n=10).

These data show that nitric oxide can produce almost total smooth muscle relaxation in concentrations which have little effect on membrane potential. However, as high concentrations of nitroprusside induce hyperpolarization, and as L-NMMA reduces both the hyperpolarization and the relaxation to acetylcholine, the hyperpolarization to nitric oxide may contribute to both of these acetylcholine-induced responses.

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412P INDOMETHACIN BLOCKS THE ENDOTHELIUM-DEPENDENT CONTRACTION TO 5-HT IN THE RABBIT ISOLATED BASILAR ARTERY

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Endothelial cells appear to modulate the constrictor action of 5-hydroxytryptamine in the rabbit basilar artery in a complex way. Smooth muscle contraction to 5-hydroxytryptamine can be increased by removal of the endothelium, inferring either spontaneous or stimulated release of EDRF (Garland, 1987). However, in the presence of partial smooth muscle contraction, both 5-hydroxytryptamine and 5-carboxamidotryptamine can stimulate additional, endothelium-dependent contraction, possibly by releasing a constrictor factor(s) from the endothelium (Clark & Garland, 1990). In canine cerebral arteries, endothelial cells exert a constrictor action on the smooth muscle in response to a number of stimuli. In some cases, this apparently involves metabolites of arachidonic acid (Shirahase et al, 1987).

The present study investigated a possible involvement by products of cyclo-oxygenase activity, in endothelium dependent contraction to 5-hydroxytryptamine in the rabbit basilar artery. Segments of basilar artery (2mm in length) were mounted horizontally in physiological salt solution at 37°C, allowing changes in smooth muscle tension to be measured. 5-hydroxytryptamine (1nM-100μM) produced additional, endothelium dependent contraction in segments which were submaximally contracted in the presence of 1μM histamine. Contraction was initiated with concentrations of 5-hydroxytryptamine in excess of 1nM, and reached a maximum, equivalent to a further 22.3% contraction, in the presence of 1μM (n=6). Incubation of the artery segments with the cyclo-oxygenase inhibitor, indomethacin (3μM), for 30 minutes before the addition of histamine and then 5-hydroxytryptamine, did not alter the contraction to histamine (12.4 ± 1.4 vs 13.8 ± 0.8 mN), but completely abolished the additional contractions to 5-hydroxytryptamine, across the entire dose range. However, a time-dependent fade of the histamine contraction did not occur, indicating that 5-hydroxytryptamine was still directly stimulating the smooth muscle cells.

The ability of indomethacin to block endothelium-dependent contractions to 5-hydroxytryptamine, suggests that these responses may be mediated by a metabolite of arachidonic acid.

This work was supported by the Wellcome Trust and the SERC.

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413P HUMAN BRAIN CAPILLARY ENDOTHELIAL CELLS RESPOND DIFFERENTLY TO THOSE OF THE BOVINE AORTA TO P_2-PURINERGIC STIMULATION

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Endothelial cells from different domains of the vasculature have different functional responses. Previously we have shown heterogeneous responses and rank order of agonist potencies to a range of P_2-purinergic agonists in adrenal medulla and aortic endothelial cells (Allsup & Boarder, 1990). We have also shown that [$^{32}P_i$] labelled aortic endothelial cells accumulate [^{32}P]phosphatidic acid (PA) on stimulation of P_2-purinergic receptors. In this study we have used [^{32}P]PA accumulation and generation of total and individual IP's as indices of purinergic stimulation of human brain capillary endothelial cells (HBEC) and bovine aortic endothelial cells (BAEC).

BAEC were prepared by collagenase treatment of fresh aortae and cultivated in MEM-Dval with 10% foetal calf serum. HBEC were prepared by immunoabsorbance using an endothelium specific antibody (Clarke & West in preparation) and grown in M199 with 15% foetal calf serum. Total IP accumulation was measured in confluent cells in 24 well dishes pre- labelled for 48h with myo-[^{3}H] inositol. Cells were stimulated for 30 min in the presence of 10mM LiCl. Generation of individual IP's separated by anion exchange chromatography was also measured. PA accumulation was measured in confluent cells in 24 well dishes loaded for 24h with [$^{32}P_i$]. Cells were chloroform extracted following 3 min stimulation and [^{32}P]PA was separated by thin layer chromatography. Fold/Basal stimulations of total IP's and [^{32}P]PA for the two cell types are shown below:

	HBEC		BAEC	
AGONIST (30μM)	IP	[^{32}P]PA	IP	[^{32}P]PA
2-methylthio ATP (2MeSATP)	1.18 ± 0.08	1.05 ± 0.09	1.70 ± 0.19	4.04 ± 0.40
ATPγS	1.04 ± 0.09	1.03 ± 0.11	6.32 ± 0.70	6.51 ± 0.95
β,γ-methylene ATP (β,γ-MethATP)	1.14 ± 0.16	1.16 ± 0.02	0.98 ± 0.10	1.27 ± 0.21
UTP	1.28 ± 0.13	1.36 ± 0.11	1.90 ± 0.60	4.56 ± 0.63
ATP	-	1.35 ± 0.02	-	3.93 ± 0.24
Mean ± SE	n = 4	n = 3	n = 4	n = 3

Separation of IP's from BAEC gave clear IP_1, IP_2 and IP_3 peaks and a sustained IP_4 rise on ATP stimulation, whereas no clear pattern of stimulation was observed in HBEC. The modest responses of HBEC to only UTP and ATP markedly contrast with the responses of BAEC to UTP, ATP and 2MeSATP and the strong response to ATPγS. These data suggest that HBEC may have a different purinergic sensitivity related to their unique role in vivo.

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414P BINDING OF ADENOSINE-5'-0-2-THIO[^{35}S]-DIPHOSPHATE TO CULTURED ENDOTHELIAL CELLS: USE OF AN EQUILIBRIUM PROCEDURE

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Receptors for adenosine-5'-triphosphate (ATP) and adenosine-5'-diphosphate (ADP) (P_2-purinergic receptors) have been divided into P2X and P2Y subtypes on the basis of rank orders of agonist potency and functional studies: in recent publications further subtypes for ATP/ADP receptors have been proposed (O'Conner et al., 1991). Binding of adenosine-5'-O-2-thio[^{35}S]diphosphate ([^{35}S]ADPβS) has been used to characterise P_{2Y}-receptors in turkey erythrocyte membrane preparations (Cooper et al., 1989). Preliminary studies showed that binding to cell monolayers was with low affinity (i.e. μM range), so we devised a simple equilibrium binding procedure to preclude loss of bound ligand expected during separation of bound from free.

Bovine aortic endotehlial cells were grown to confluence in 24-well multiwells. Cells were incubated with HEPES buffered (pH 7.4) balanced salt solution with no magnesium, at 4°C for 4h in the presence of 0.1 to 0.5nM [^{35}S]ADPβS (NEN) (specific activity, 1000-1200 Ci/mmol). An aliquot of the incubate was removed and kept and the incubation terminated, and cells solubilised with the addition of 0.2M NaOH. Radioactivity in both samples was measured and the difference between the two used to determine the amount of bound radioligand.

Saturation binding curves were generated using increasing concentrations of unlabelled ADPβs and isotope dilution analysis. Iterative curve fitting resolved two binding sites (apparent K_D 36.6 ± 8.0 nM, B_{max} 69.4 ± 4 pmol/mg protein; apparent K_D 6330 ± 270 nM, B_{max} 1844.3 ± 287 pmol/mg protein). Generation of competition binding curves with several ATP analogues gave a rank order of inhibition of total binding of: ADPβS (IC_{50} 13.3 ± 2.0 μM) > ATPγS (IC_{50} 36.6 ± 3.3 μM) > 2-methylthio ATP (80% inhibition of binding) > β,γ-methylene ATP (51% inhibition of binding). Non-specific binding was determined in the presence of 100μM ATPγS. Neither 2-methylthio ATP or β,γ-methylene ATP were able to induce complete inhibition of [^{35}S]ADPβS binding.

This rank order of inhibition of binding corresponds to none of the described agonist potency profiles of the various proposed P_2 or related receptors. Thus, while these studies show that a simple equilibrium procedure can be used to study [^{35}S]ADPβS binding to cultured endothelial cells, it remains to be established whether this binding corresponds to a functional cell surface receptor.

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415P [^{3}H]-ADRENALINE DOES NOT LABEL AN α_{2B}-ADRENOCEPTOR ON RAT NEONATAL LUNG

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α_2-Adrenoceptors have been subclassified into 3 or perhaps 4 subtypes based on the rank order of affinity of a number of compounds for [^{3}H]-yohimbine binding in a variety of tissues. The subtype on rat neonatal lung membranes has been classified as α_{2B} based on the relatively high affinity of prazosin and low affinity of oxymetazoline (Bylund et al., 1988). However, the affinity of prazosin for [^{3}H]-adrenaline binding in the rat neonatal lung is very low (Kahn et al., 1982). The highly selective α_2 antagonist ligand [^{3}H]-RS-15385-197 is non-selective between α_2-adrenoceptor subtypes and labels a site on rat neonatal lung which has high affinity for prazosin (MacKinnon et al., 1991). It was the object of this study to characterise and compare the site labelled by [^{3}H]-adrenaline and [^{3}H]-RS-15385-197 on rat neonatal lung membranes.

[^{3}H]-RS-15385-197 binding to rat neonatal lung membranes was carried out as previously described (MacKinnon et al., 1991). [^{3}H]-adrenaline binding was carried out in a 50 mM Tris HCl buffer containing, 0.3 mM ascorbate, 5 mM $MgCl_2$, 0.8 mM dithiothreitol and 0.1 mM catechol which minimises degradation of the radioligand (Se'nard et al., 1988). The buffer also contained 10 nM propranolol to prevent binding to β-adrenoceptors.

[^{3}H]-RS-15385-197 labelled a single population of high affinity sites in the rat neonatal lung; Kd = 0.08±0.02 nM, Bmax = 174±11 fmoles/mg protein. (n=5). [^{3}H]-Adrenaline labelled fewer sites than [^{3}H]-RS-15385-197 and had lower affinity (Bmax = 77.7±11 fmoles/mg protein, Kd = 3.03±0.44 nM, n=5). The α_{2B} selective compounds prazosin and imiloxan showed high affinity for [^{3}H]-RS-15385-197 binding (pKi = 7.49±0.12 and 7.36±0.02 respectively, n=3) but low affinity for [^{3}H]-adrenaline binding (pKi = 5.68±0.16 and 5.61±0.12, n=3). In contrast the α_{2A} selective compound oxymetazoline showed high affinity for [^{3}H]-adrenaline binding (pKi = 8.22±0.09, n=3), but low affinity for [^{3}H]-RS-15385-197 binding (pKi = 6.91±0.04, n=3). Correlation studies for a number of compounds revealed that α_2 agonists showed higher affinity for [^{3}H]-adrenaline binding while the antagonists displayed higher affinity for [^{3}H]-RS-15385-197 binding. These results suggest that the site labelled by [^{3}H]-adrenaline on the neonatal rat lung does not show the characteristics of an α_{2B}-adrenoceptor subtype. The α_{2B} subtype may represent a low affinity agonist state of the α_2-adrenoceptor.

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416P NEURALLY-EVOKED RESPONSES OF HUMAN ISOLATED RESISTANCE ARTERIES ARE MEDIATED BY BOTH α_1- AND α_2-ADRENOCEPTORS

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The presence of postjunctional α_1- and α_2-adrenoceptors has been demonstrated in human subcutaneous resistance arteries using exogenously applied α-agonists and antagonists (Nielsen *et al*, 1990). This study is investigating the contribution of α_1- and α_2-adrenoceptors to the neurally-evoked response of human subcutaneous resistance arteries.

Electrical field stimulation was applied to human subcutaneous resistance arteries (84μm - 562μm I.D.) mounted as ring segments in physiological saline solution at 37°C, bubbled with 95% oxygen and 5% carbon dioxide. The vessels were then 'normalised' as described by Mulvany & Halpern (1977). Electrodes were placed 2-3 mm from each side of the vessels. Prior to stimulation the vessels were exposed three times to 10μM noradrenaline (NA) to assess the maximal adrenergic response. Stimulation was applied in two ways: (1) continuously in the form of two frequency response curves (FRCs) at 16 Volts with a pulse width of 0.3 msec, from 0.5 Hz increasing to 8 Hz, the second FRC performed in the presence of 0.1μM or 1μM prazosin (an α_1-antagonist) or rauwolscine (an α_2-antagonist) or in combination; (2) intermittent stimulation at 5-11 Volts, 0.3 msec width, and 16 Hz for 5 sec every 2 min for 50 min, with increasing concentrations of either an α_2-adrenoceptor antagonist (yohimbine or rauwolscine), or an α_1-adrenoceptor antagonist (doxazosin or prazosin) of 1nM, 10nM and 100nM, introduced into the bath after 20, 30 and 40 min. The stimulation parameters were checked with tetrodotoxin (1μM) to ensure that the response achieved was neurogenic.

Continuous stimulation. The maximum control response of the 2nd FRC was 100%±5 of the 1st FRC. 0.1μM Prazosin inhibited the maximum response of the 2nd FRC by 52%±5 and 0.1μM rauwolscine by 45%±7. Combined rauwolscine and prazosin (0.1μM) caused a 72%±6 inhibition. 1μM Rauwolscine inhibited the 2nd FRC by 72%±5, 1μM prazosin by 84%±7, combined rauwolscine and prazosin by 94%±3. **Intermittent stimulation.** Yohimbine (100nM) blocked the intermittent stimulation by 44%±11, rauwolscine (100nM) by 82%±10, doxazosin (100nM) by 33%±10, and prazosin (100nM) by 72%±2.

We conclude that the contractile response to electrical field stimulation is mediated through both α_1- and α_2-adrenoceptors. As the electrical stimulation was within neuronal parameters, both α_1- and α_2-adrenoceptors may be activated by the neuronal release of the nerve transmitter NA.

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417P EFFECT OF U46619 AND OXYGEN IN THE SURFACE CHORIONIC AND STEM VILLUS ARTERIES OF THE HUMAN PLACENTA

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We have previously demonstrated in the human umbilical artery that increasing oxygen tension above 16mmHg produced a transient contraction due to release of thromboxane (Templeton *et al*, 1991). In this study we have examined the effects of oxygen *per se* and the thromboxane mimetic U46619 on various generations of artery in the placenta to determine if the effect of oxygen prevails into the placenta.

Rings(2-3mm) of primary and secondary surface chorionic arteries were set-up between two wire hooks one of which was fixed, the other attached to an isometric transducer, in Krebs' at 37°C bubbled with 2.5% O_2/8%CO_2 (bath O_2 tension 16.4±0.5mmHg, CO_2 35.5±0.6mmHg)balance N_2 under 1.5g tension. Tertiary surface chorionic (150-250μm internal diameter) and stem villus arteries (100-180μm internal diameter) were set-up on a wire myograph and normalised to 0.9 of L_{100} (Mulvany & Halpern 1977). After 1 hour equilibration all tissues were first exposed to 20% oxygen for 5 minutes then returned to 2.5%. Thereafter GR32191B(10nM-1μM) a selective thromboxane receptor antagonist was added for 40 minutes before exposure to 20% oxygen was repeated. Tissues were then returned to 2.5% oxygen and in the presence of the antagonist a cumulative concentration response curve to U46619 was constructed. Primary and secondary surface chorionic arteries contracted in response to 20% oxygen(n=6). This effect was antagonised by GR32191B (10nM-1μM). Tertiary surface chorionic vessels mounted on the myograph also contracted in response to 20% oxygen, a response inhibited by flurbiprofen (0.1μM) (n=2) or GR32191B (0.1μM) (n=4). In contrast the stem villus arteries did not contract in response to this elevated oxygen tension (n=10). All vessels contracted in a concentration-dependent manner to U46619, an effect antagonised by GR32191B. Results are detailed in the table.

TABLE 1

Vessel	Max Response(mg.wt)	pD_2	pA_2 (GR32191B)	Slope
Primary	2458±340	7.89±0.08	8.29(8.19-8.41)	0.99(0.93-1.07)
Secondary	2330±160	7.85±0.09	8.44(8.31-8.57)	0.88(0.81-0.95)
Tertiary	450±89	7.76±0.09	--	--
Stem Villous	490±140	7.78±0.13	--	--

This table shows the maximum response to U46619 along with pD_2 values. In addition it shows the pA_2 value and slope with 95% confidence limits for GR32191B in primary and secondary surface chorionic arteries. Values represent mean ±sem (n=4-6).

These results show a similar sensitivity to U46619 throughout the placental artery tree, with a 0.5 log unit decrease in sensitivity compared with umbilical artery. However the vessels below the chorionic plate do not contract to oxygen which may indicate a lack of the enzyme responsible for the production of thromboxane, as the receptors are present and functional.

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418P PHARMACOLOGICAL CHARACTERISATION OF THE PROSTANOID RECEPTORS IN THE RABBIT ISOLATED EAR ARTERY

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De la Lande and Rand (1965) first introduced the rabbit ear artery (RbEA) as a simple nerve-blood vessel preparation, and it has been used extensively for investigating the effects of novel drugs on isolated vascular smooth muscle. Prostanoids are well known for both their vasodilator and vasoconstrictor activity, and in the present study we have investigated the effects of a wide range of prostanoid agonists on isolated ring preparations of the RbEA in order to characterize the receptors mediating prostanoid-induced responses.

Ear arteries were dissected from New Zealand White rabbits (1.5-2.0 Kg), and were cut into rings, approximately 3mm in length. These arterial rings were suspended in organ baths containing oxygenated (95% O_2/5% CO_2) modified Krebs solution (+ indomethacin 2.8μM) maintained at 37°C.

PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$ and the selective TP-receptor agonist, U46619,(0.001-50μM), were evaluated for their ability to relax tone induced by phenylephrine (PE, 0.1-1μM). PGE_2 and PGI_2 both caused concentration-related relaxation, whereas PGD_2, $PGF_{2\alpha}$ and U46619 had little or no relaxant activity at concentrations up to 50μM. We went on to evaluate a range of selective EP-, IP- and DP- receptor agonists. AH13205 and butaprost (EP_2-selective), misoprostol (EP_2/EP_3), cicaprost (IP) and iloprost (EP_1/IP) all caused concentration-related relaxation of PE-induced tone. However, enprostil (EP_3/EP_1) and BW245C (DP) were weak agonists and produced significantly lower maximal responses. Sulprostone (EP_1/EP_3) had no relaxant activity at concentrations up to 50μM. The equipotent concentration (EPC) values for these agonists relative to PGE_2 are shown in the Table.

Prostanoid	EPC (PGE_2=1)	Prostanoid	EPC (PGE_2=1)
PGE_2	EC_{50} = 80 (40 - 160)nM	Iloprost	4.1 (2.1-8.2)
PGI_2	7.2(3.2- 16.2)	Butaprost	16 [6 - 36]
PGD_2	> 1000	AH13205X	50 (32 - 76)
$PGF_{2\alpha}$	> 700	Enprostil	215[90 - 340]
Misoprostol	0.9(0.3 - 2.9)	BW245C	302(230- 397)
Cicaprost	1.2(0.2 - 5.9)	Sulprostone	> 770

Values are geometric means (95% confidence limits) where n ≥ 4, or [range] where n=3.

These data suggest that the RbEA contains relaxant prostanoid EP- and IP- receptors, and the similarity of the rank order of potency of EP-receptor agonists to that previously described for the cat trachea (Coleman et al, 1987) an EP_2- receptor containing preparation, suggests that the receptors are of the EP_2 subtype. Thus, the RbEA may represent a suitable preparation on which to evaluate potential EP_2- and IP- receptor agonists and antagonists.

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419P ENDOTHELIN-1-INDUCED VASOCONSTRICTION AND HYPOXIA IN THE RABBIT PULMONARY ARTERY

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We have previously shown that the endothelium-dependent *in vitro* vasoconstrictor response to acute hypoxia in the rabbit pulmonary artery was inhibited by the nitric oxide (NO) synthase inhibitor L-NAME. It was not inhibited by the calcium channel blocker nifedipine but was reduced in Ca^{2+}-free Krebs (MacLean & McGrath, 1991). Here, we have conducted a comparable study on vasoconstriction induced by the vasoconstrictor peptide endothelin-1 (Et-1) to investigate if it is involved in acute hypoxic-induced vasoconstriction (HPV).

Isolated rings (3-5mm) of the first left branch of the pulmonary artery were dissected from NZW rabbits (2-3 kg), newly killed by stunning and cervical dislocation. Rings were suspended in organ baths containing Krebs saline at 37°C under 2g force and gassed with 5% O_2, 6% CO_2 balance N_2 (bath O_2 tension=45.3±0.5mmHg, CO_2=35.6±0.4mmHg, pH=7.39±0.04, n=6). The effect of hypoxia was studied by gassing with 0% O_2, 6% CO_2 balance N_2 (bath O_2 tension=8±0.6mmHg, CO_2=35.8±0.5mmHg, pH=7.37±0.01, n=6).
Dose-response curves to Et-1 were constructed in the absence and presence of hypoxia, L-NAME (10^{-4}M for 20min), nifedipine (10^{-5}M for 45 min) and extracellular calcium (substituting normal Krebs for Ca^{2+}-free Krebs plus 0.05mM EGTA).

Table 1 -Log concentration of the EC_{50} and maximum responses (tension [mg wt]) to Et-1: effect of L-NAME, nifedipine, hypoxia and Ca^{2+}-free Krebs

	CONTROL GROUPS		TEST GROUPS		
Group test	EC_{50} (-log M)	Maximum response	EC_{50} (-log M)	Maximum response	n
Ca^{2+}-free Krebs	8.3 ± 0.1	2170 ± 240	8.4 ± 0.5	840 ± 174**	5
Nifedipine	8.7 ± 0.3	1850 ± 338	8.2 ± 0.1†	1875 ± 295	5
L-NAME	8.5 ± 0.2	2075 ± 193	9.0 ± 0.2†	2488 ± 249**	5
Hypoxia	8.7 ± 0.1	1960 ± 204	8.4 ± 0.1	2420 ± 242*	5

Paired Student's *t* test: EC_{50} for control vs. test group, † $P<0.05$; maximum response for control vs. test group, *$P<0.05$, **$P<0.01$.
Table 1 shows that reduction of extracellular Ca^{2+} reduced the maximum responses to Et-1. Nifedipine reduced the potency of Et-1 while L-NAME increased both the potency of, and the maximum response to, Et-1. These actions were in contrast to the lack of nifedipine effect, and the inhibitory action of, L-NAME on HPV shown in our previous study which suggested that hypoxia inhibits NO release (MacLean & McGrath, 1991). Here, hypoxia itself increased the maximum response to Et-1. Hence, the results do not suggest that Et-1 is involved in the HPV induced by acute hypoxia. The potentiating effect of L-NAME suggests that Et-1 may release NO in the rabbit pulmonary artery consistent with its effect in the rabbit aorta (Warner *et.al.*, 1989). If acute hypoxia inhibits NO release in the rabbit pulmonary artery *in vitro* as suggested by our previous study, this would contribute to its potentiation of Et-1-induced vasoconstriction observed here.

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420P N^G-NITRO-L-ARGININE (NOLA) POTENTIATES 5-HT-, BRADYKININ (BK)- AND ENDOTHELIN-1 (ET1)-INDUCED VASOCONSTRICTION IN THE HUMAN FETAL PLACENTA

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We have previously reported evidence supportive of a role for endothelium-derived relaxing factor (EDRF) in the maintenance of low fetal placental vascular resistance (Gude et al., 1990). This study investigates the role of EDRF in modulating the responses of the fetal placental vessels to certain vasoconstrictors. Human placental lobules were bilaterally perfused in vitro with Krebs solution at flow rates of 5ml/min and fetal inflow perfusion pressure was recorded. Basal pressure 1h after commencement of perfusion was 32.1 ± 1.2mmHg (n=17). Infusion of the EDRF biosynthesis inhibitor NOLA (60μM) caused a significant increase in perfusion pressure of 50.5 ± 6.8mmHg (n=16). Increases in perfusion pressure were measured in response to a series of increasing doses of 5HT, ET1 or BK in the presence or absence of infused NOLA (60μM). NOLA significantly potentiated the responses to all three vasoconstrictors as judged by either an increase in the maximal response or a decrease in the EC_{50}.

Table 1

	Maximum pressure increase (mmHg)		EC_{50} (nM)	
	without NOLA	with NOLA	without NOLA	with NOLA
5HT	26.6±8.5, n=7	80.4±21.2*,n=5	492.9±110.2	92.1±13.9*
ET1	134.7±29.9, n=6	171.5±14.5, n=5	15.9±0.9	2.1±0.6*
BK	5.9±2.0, n=7	27.5±8.6*, n=4	143.7±42.5	167.9±52.3

* Significantly different from without NOLA, unpaired t-test, $P<0.05$.

These data suggest that EDRF is released by the fetal vessels of the human placenta and that it may attenuate responses to a number of vasoconstrictors.

Gude, N.M., King, R.G. & Brennecke, S.P. (1990) Lancet. 336, 1589-90.

421P THE EFFECT OF ENDOTHELIN-1 ON ISOMETRIC TENSION AND ^{86}RUBIDIUM EFFLUX IN RAT AORTA

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We have investigated the effects of endothelin-1 (ET-1) on isometric tension in the isolated superfused rat aorta and measured the concomitant efflux of pre-loaded ^{86}Rubidium, in comparison with 40mM K^+ (K^+) and noradrenaline (NA) using published methodology (Martin & Gordon, 1983).

Thoracic aortae were removed from male Wistar rats, spirally cut into 2mm x 2cm strips and de-endothelialized. These strips were incubated with 10μCi ml^{-1} ^{86}RbCl for 2 hours in Krebs solution at 37°C. Strips were then mounted for recording of isometric tension at a basal tension of 1g and superfused with Krebs solution at 2ml min^{-1} at 37°C. After an equilibration period of 20min, during which time the rate of ^{86}Rb efflux becomes constant, 1min fractions were collected. At the end of the experiment ^{86}Rb in these fractions and in the tissue was counted in an LKB gamma counter. Responses to 5 minute infusions of 10^{-7}M NA and 40mM KCl or 10 minute infusions of 10^{-8}M ET-1 were recorded. In separate experiments the tissue was superfused with calcium-free Krebs containing 10^{-3}M ethylene glycol-bis (β aminoethyl ether) N,N,N',N'-tetra-acetic acid (EGTA) with or without 10^{-5}M ryanodine. A desaturation plot of tissue ^{86}Rb content was constructed by adding (in reverse order) the radioactivity in each fraction to that remaining in the tissue at the end of the experiment. The rate constant (R) for ^{86}Rb loss was then calculated.

K^+, NA and ET-1 contracted the isolated rat aorta (0.57±0.06, 0.68±0.07 and 0.86±0.10 gram tension respectively) and caused the efflux of ^{86}Rb (5.8±1.0, 5.2±1.0 and 4.3±0.8 increase in R respectively). Rubidium efflux was correlated with the magnitude and time course of contraction. Therefore the prolonged contractile response to ET-1 was mirrored by a prolonged ^{86}Rb efflux. In calcium-free Krebs the responses to all three agonists were greatly reduced (0.08±0.00, 0.08±0.02 and 0.13±0.02 gram tesnsion respectively); ^{86}Rb efflux in response to NA and ET-1 was abolished, but after K^+ ^{86}Rb efflux was as large as or larger than in normal Krebs (R = 11.0±1.0). The addition of ryanodine to calcium-free Krebs had no additional effect either on contraction or ^{86}Rb efflux.

The results confirm previous reports that the response to ET-1 is largely dependent on the presence of extracellular calcium, though the release of intracellular calcium from ryanodine-insensitive stores also contributes to the response. The efflux of rubidium from rat aorta in response to ET-1 appears to be similar to that described for the response to NA both in this study and in rabbit aorta (Martin & Gordon, 1983). That is, ^{86}Rb efflux is correlated with the contractile response and both are dependent on the presence of extracellular calcium. However, intracellular calcium stores are capable of initiating a small contraction in response to ET-1, but not capable of inducing ^{86}Rb efflux. For both NA and ET-1 rubidium efflux is therefore via calcium-activated potassium channels. The effect of ET-1 contrasts with that of K^+, which also stimulates a calcium-dependent contraction, but which induces rubidium efflux through a different mechanism, presumably via voltage operated channels.

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422P DECREASED RESPONSES TO PLATELET ACTIVATING FACTOR (PAF), ENDOTHELIN-1 (ET-1) AND ANGIOTENSIN II (A II) IN TUMOUR BLOOD VESSELS IN MICE

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Blood vessels induced by tumour growth are morphologically different from normal blood vessels (Jain, 1989). We have looked for pharmacological differences between normal and tumour induced blood vessels, using a model of angiogenesis in mice modified from that originally described by Andrade et al (1987). Discs of polyether-polyurethane sponge (4mm thick, 8mm radius) were implanted subcutaneously on the backs of anaesthetized male mice (Balb/C, 22-28g body weight) and subsequent vascularization of the sponge assayed by the washout of ^{133}Xe injected into the implant. Radioactivity over the implant was measured in anaesthetized mice for at least 6min after ^{133}Xe injection and the rate of washout expressed as the T1/2 (time for radioactivity to fall to 50% of the initial value). Between 1 and 4 days after implantation, T1/2 was stable at 24±6min (mean±S.D., n > 30). By 7 days, T1/2 had fallen to 15±3min and by 13 days, to 6.5±2.5min, within the range of T1/2 for normal untreated skin (5±1min). Histological examination showed a good correlation between morphological signs of neovascularization and the reduction in T1/2. Tumour cells (10^6 of Colon 26 cells) were injected into sponges 2 days after implantation and in these implants angiogenesis was accelerated; by 7 days, T1/2 was 8±2min and 7±2min by 10 days.

Responses of the blood vessels in control and tumour bearing implants were measured at 14 days when normal T1/2 values had been attained. In control implants, all three agonists increased T1/2, i.e. decreased blood flow, from 6±2min to 8±1min with PAF (0.5μg); to 25±6min with ET-1 (5pmole); to 13±1min with A II (50ng). These responses were comparable to those obtained in normal skin vessels adjacent to the implant. In tumour bearing implants, the vessels in the implant were less responsive to the agonists even at higher doses; T1/2, untreated 5±2min; PAF (2μg), 4±1min; ET-1 (50pmole), 13±5min; A II (500ng) 19±7min (n>7 for each). From these results we conclude that the model provides a quantitative assessment of vasoactive agents in a newly formed vascular bed and that tumour induced blood vessels are pharmacologically different from host vessels.

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423P CARDIOVASCULAR EFFECTS OF THE ANGIOTENSIN RECEPTOR ANTAGONIST, GR117289, IN CONSCIOUS RENAL HYPERTENSIVE AND NORMOTENSIVE RATS

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GR117289 is a potent, specific antagonist of angiotensin II (Ang II) *in vitro* (Robertson et al., 1991). We have examined its antihypertensive effect in rats (male AH/A) made hypertensive (diastolic blood pressure >140mmHg) by activation of the renin-angiotensin system after left renal artery ligation (RALH rat), 6 days previously (Cangiano et al., 1979). Furthermore, the ability of GR117289 to displace Ang II dose-pressor response curves was investigated using normotensive rats.

Blood pressure was measured from carotid artery catheters and heart rate was derived from the pressure signal. Ang II was injected intra-arterially and GR117289 was administered intra-arterially or orally. GR117289 (0.3-3mg kg^{-1} ia, 0.3-10mg kg^{-1} po) caused dose-dependent, long-lasting decreases in blood pressure (Table 1) in RALH rats; heart rate was little changed (<10%). In normotensive rats, GR117289 (3mg kg^{-1} ia or po) had minimal effects on blood pressure (<10% change) but caused parallel, rightward displacements of Ang II dose-pressor response curves (Table 1). In contrast, GR117289 (3mg kg^{-1} po) did not affect vasopressor responses to phenylephrine (1-10μg kg^{-1} ia).

Table 1 **Antihypertensive and Ang II Antagonist Activities of GR117289 in Rats**

	HOURS AFTER IA ADMINISTRATION						HOURS AFTER PO ADMINISTRATION					
GR117289 [mg kg^{-1}]	1	3	5	7	24	48	1	3	5	7	24	48
	Changes in Diastolic Blood Pressure [mmHg; arithmetic mean; n=5-6 RALH rats]											
0.3	-17	-32	-32	-35	- 4	0	3	6	- 3	0	- 4	0
1	-33	-38	-40	-43	-18	7	-1	- 7	-20	-20	-15	- 7
3	-41	-55	-64	-70	-53	-18	-7	-15	-36	-42	-44	- 7
10	-	-	Not	Tested	-	-	-14	-38	-55	-58	-56	-19
	Ang II Dose-Ratio [geometric mean (± 95% confidence interval); n=5-6 normotensive rats]											
3	111	59	37	28	22	5	3	4	7	7	2	1
	(29-423)	(37-95)	(24-57)	(16-50)	(5-88)	(2-13)	(2-7)	(2-7)	(4-12)	(4-11)	(0.8-4)	(0.4-2)

In conclusion GR117289 is a potent, specific, long-lasting and orally active angiotensin antagonist in normotensive rats and reduces blood pressure in RALH rats.

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Robertson, M.J., Middlemiss, D., Ross, B.C., Drew, G.M., Scopes, D.I.C. & Dowle, M.D. **This Meeting.**

424P CARDIOVASCULAR EFFECTS OF THE ANGIOTENSIN RECEPTOR ANTAGONIST, GR117289, IN CONSCIOUS DOGS AND MARMOSETS

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GR117289 is a potent, specific antagonist at angiotensin AT_1 receptors *in vitro* and *in vivo* and produces a long-lasting reduction in blood pressure after both intra-arterial and oral administration to renal hypertensive rats (Robertson et al., 1991; Hilditch et al., 1991). We have now examined its cardiovascular profile and angiotensin receptor blocking activity in conscious beagle dogs and marmosets.

Cannulae were inserted in a carotid artery loop and jugular vein (dog) or implanted in a femoral artery and vein (marmoset) for resting diastolic blood pressure (DBP) recording and drug administration, respectively. Heart rate was derived from the pressure signal. Incremental doses of angiotensin II (Ang II) were administered (iv) before and at various times up to 24h after administration of GR117289, and the displacement of the pressor response curve expressed as an Ang II dose-ratio at each time point. Ang II (10-300 or 15-150ng kg^{-1}) elicited dose-dependent increases in DBP in dogs and marmosets. GR117289 (3mg kg^{-1} iv or po) had minimal effects on DBP or heart rate, but produced parallel, rightward displacements of Ang II dose-pressor response curves (Table 1). In contrast to its minimal effect on DBP in sodium-replete dogs, GR117289 (3mg kg^{-1} iv) reduced DBP ($p<0.05$) in sodium-deplete dogs (frusemide, 5mg kg^{-1} iv; 18 and 1h pretreatment) by 20-23 mmHg for at least 5h after administration.

Table 1 **Cardiovascular Effects of GR117289 (3mg kg^{-1}) in Conscious Dogs (n=4) and Marmosets (n=4-6).**

		HOURS AFTER ADMINISTRATION									
		DOG					MARMOSET				
	ROUTE	0	1	3	5	24	0	1	3	5	24
DBP mmHg	IV	73	66	77	75	68	127	117	116	117	113
(arithmetic mean)	PO	76	67	73	73	65	130	126	124	122	123
ANG II DOSE-RATIO	IV	1	32	3	2	1	1	256	27	17	2
(geometric mean)	PO	1	12	5	4	1	1	9	7	6	1

In conclusion, GR117289 has minimal effects on resting diastolic blood pressure or heart rate in conscious normotensive dogs and marmosets at a dose that inhibits vasopressor responses to Ang II. However, following sodium depletion, GR117289 reduces resting diastolic blood pressure in the conscious dog to a greater extent than in sodium-replete animals.

Hilditch, A., Akers, J.S., Travers, A., Hunt, A.A.E., Robertson, M.J., Drew, G.M., Middlemiss, D. & Ross, B.C. **This Meeting.**
Robertson, M.J., Middlemiss D., Ross, B.C., Drew, G.M., Scopes, D.I.C. & Dowle, M.D. **This Meeting.**

425P THE INTERACTION OF GR117289 WITH THE ANGIOTENSIN AT_1 AND AT_2 BINDING SITES

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Recent studies have suggested that angiotensin (AII) receptors can be divided into AT_1 and AT_2 subtypes on the basis of their affinity for peptide and non-peptide antagonists (Rogg et al., 1990). In the present study we report on the interaction of a novel non-peptide antagonist, GR117289 (Robertson et al., this meeting), with the AT_1 and AT_2 binding sites identified *in vitro*.

Binding studies on the AT_1 and AT_2 sites were conducted as described previously (Michel & Barnes, 1991). The AT_1 sites in the rat liver and the AT_2 sites in bovine cerebellum were labelled using either [^{3}H]AII or [^{125}I]sar^1-ile^8-AII ([^{125}I]-sarile). Assays were conducted using a 50mM Tris, 100mM NaCl, 10mM $MgCl_2$, 1mM Na_2EDTA, 0.1% BSA, 1mM bacitracin buffer, pH 7.4. Incubations were for 90mins at 22°C and were terminated by vacuum filtration.

In competition binding studies using [^{3}H]AII, GR117289 was a potent competitor for the AT_1 binding sites in the rat liver [pK_i = 7.5 ± 0.1; Hill coefficient (nH) = 1.11, n=5] and was essentially devoid of affinity for the AT_2 sites in bovine cerebellum (pK_i < 5 n=2). In additional studies conducted using [^{125}I]-sarile, GR117289 displayed pIC_{50} values (mean ± S.E.M of n=3-4) of 7.8 ± 0.2, 7.4 ± 0.1 and 7.8 ± 0.1 for the AT_1 sites in rat uterus, adrenal and liver, respectively. The pK_i value obtained at the rat liver AT_1 site (7.5 vs [^{3}H]AII) was substantially lower than the pK_B of 9.8 obtained in functional studies on the AT_1 receptor in rabbit aorta (Robertson et al., this meeting). This was probably due to GR117289 binding to the BSA in the assay buffer since reducing the BSA level from 0.1% to 0.001% resulted in a 30 fold increase in the potency of GR117289 (pK_i = 9.0 ± 0.1, nH=1.09, n=7). Under these conditions GR117289 was 7.9 fold more potent than DuP 753 (pK_i = 8.1 ± 0.1; n=6). In the competition studies, the nH for the GR117289 competition curve was not significantly different from unity suggesting that the compound was a competitive antagonist. However, in saturation binding studies (conducted in 0.001% BSA containing buffer), 12nM GR117289 decreased the B_{max} by 40% and increased the K_D of [^{3}H]AII 2.3 fold. This could suggest that GR117289 is either a non-competitive/allosteric inhibitor of binding or that GR117289 dissociated slowly from the AT_1 site. An allosteric effect of GR117289 seemed unlikely since GR117289 (0.1 and 1uM) did not affect the rate of dissociation of [^{3}H]AII from rat liver AT_1 sites, as measured in isotopic dilution experiments. Since the effects of GR117289 are only slowly reversible in functional studies (Robertson et al., this meeting) it is possible that the effects of GR117289 observed in the saturation studies are related to slow dissociation kinetics.

In conclusion, the results of this study indicate that GR117289 is a potent inhibitor of AII receptor binding and is highly selective for the AT_1 over the AT_2 site. The potency of GR117289 in binding studies can be influenced by protein binding. The results obtained in the saturation studies suggest that GR117289 is a slowly-reversible AT_1 receptor antagonist.

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426P MODULATION OF POST-JUNCTIONAL ANGIOTENSIN II-MEDIATED CONTRACTIONS IN THE RABBIT DISTAL SAPHENOUS ARTERY

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In a number of tissues, pre-treatment with small doses of selected excitatory ligands, has been shown to markedly enhance some agonist-induced contractions (e.g. in rat tail artery, MacLean & McGrath, 1990). The mechanism which underlies these interactions has not however been fully established. Our previous studies have suggested a role for protein kinase C (PKC) activation, but not $[Ca^{2+}]_i$ elevation, in the enhancement, by angiotensin II (AII) and by neuropeptide Y (NPY), of post-junctional α_2-adrenoceptor-mediated responses in the rabbit distal saphenous artery (Nally & McGrath 1991). This present study examined the effects of a number of agonists on AII-mediated contractions in this artery. Evidence for the signal-transduction mechanisms underlying any interactions between these substances has been considered and compared to that observed for α_2-adrenoceptor-mediated events.
Male New Zealand White rabbits (1.5-2.5Kg) were killed by stunning followed by exsanguination. Lengths of distal saphenous artery (2-4mm) were suspended under 1g tension for isometric recording in a modified Krebs solution (for composition see Daly *et al.*, 1988). Concentration-response curves (CRC's) were constructed to AII (10^{-11}-$3x10^{-7}$M) in the presence and absence of a variety of agonists, at concentrations producing 25-30% of their maximum response. Mean pD_2 (-log concentration of the EC50) values were calculated and results expressed as % of control maximum response (control pD_2; 8.62±0.15). Number of observations (n) >7 in each case. 5-hydroxytryptamine (5-HT, 0.1μM), endothelin-1 (Et1, 1nM) and neuropeptide Y (NPY, 50nM) each evoked a significant leftward shift (mean pD_2; 10.12±0.22 (31.6 fold shift) , 10.06±0.11 (27.5 fold shift) and 10.2±0.09 (38 fold shift) respectively, $p<0.001$) of the CRCs to AII, with increased maximum contraction in each case (mean increase; 109%, 57.6% and 72.8% respectively). CRCs to AII were 'bell-shaped' in the presence of NPY. The enhancement of AII responses evoked by 5-HT and by Et1 was mimicked by the presence of KCl (30mM, pD_2; 10.16±0.11), which opens voltage dependent calcium channels, but not by the phorbol ester, phorbol 1,2-myristate 1,3-acetate (PMA, 10nM), which stimulates PKC.
The enhancement, in the rabbit distal saphenous artery, of AII-evoked contractions by 5-HT, Et1 and by KCl, is in marked contrast to the inability of these agents to alter α_2-mediated responses in this tissue (Nally & McGrath, 1991). Similarly, the lack of effect of PMA on AII-mediated responses contrasts with the enhancement of α_2-mediated responses evoked by the phorbol ester in this tissue. It seems likely therefore that in the rabbit distal saphenous artery, elevation of $[Ca^{2+}]_i$ is important in the enhancement of AII-evoked contractions but not of α_2-mediated responses, while the opposite may apply for the involvement of PKC in enhancing post-junctional responses to these agonists.

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427P A COMPARISON OF THE ACTION OF AMLODIPINE AND VERAPAMIL IN HUMAN ISOLATED RESISTANCE ARTERIES

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Amlodipine is a 1,4-dihydropyridine calcium antagonist with novel characteristics. Voltage clamp studies of isolated cardiac myocytes have indicated that the action of amlodipine shows use-dependence (Kass and Arena, 1989), in that the degree of block becomes more pronounced if the calcium channels are stimulated frequently. We have investigated whether such use-dependence of amlodipine may be demonstrated in isolated human subcutaneous resistance arteries. Amlodipine was compared with verapamil, a phenylalkylamine calcium antagonist widely reported as showing use-dependence.

Subcutaneous arteries (internal diameter 85-456μm, n=26) were obtained from tissue resected during surgery and mounted in a myograph for the measurement of isometric tension as previously described by Mulvany and Halpern (1977). Vessels were stimulated by exposure to physiological saline containing 80mM K^+ in the absence of drug (control) and in the presence of amlodipine (1μM) or verapamil (1μM). The drug concentrations used were selected as both were found to produce in the region of 50% inhibition of control. The influence of stimulation frequency on the rate of onset and magnitude of inhibition induced by drug over the period up to steady state (verapamil 10 min or amlodipine 3h) was compared. Vessels were either stimulated only once or repeatedly at intervals (verapamil 5 stimulations, amlodipine 8 stimulations) during this period. It was necessary to use separate vessels for the single and repeated exposure experiments with amlodipine due to the drug's resistance to washout. Responses were calculated as % reduction in response compared with control prior to drug application, data are means ± s.e. means. Onset data were analysed by fitting data to an exponential function by non-linear regression and the half-time of inhibition ($t_{1/2}$) calculated. Comparisons of data were made using the Student's t-test.

The apparent rate of onset of both drugs did not differ significantly between the two activation protocols. The $t_{1/2}$ for amlodipine using single exposure was 29.4±5.0 min (n=12) and 21.0±5.0 min (n=4) using repeated exposure (NS). The $t_{1/2}$ for verapamil-induced inhibition using single exposure was 3.0±0.8 min (n=4) and 1.98±0.2 min (n=6) using repeated exposure (NS). In contrast, stimulation frequency had a marked effect on the magnitude of inhibition induced by verapamil but little effect on amlodipine-induced inhibition. Verapamil produced 33.0%±3.7 (n=4) inhibition following single exposure and 62.3%±3.1 (n=6) inhibition on repeated exposure ($p<0.01$), whereas amlodipine produced 48.0%±2.0 (n=12) inhibition on single exposure and 53.6%±5.5 (n=4) inhibition on repeated exposure (NS).

In conclusion, verapamil displays use-dependence in that inhibition is increased when arteries are activated frequently, whereas amlodipine displays little or no use-dependence in isolated human resistance arteries.

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428P VOLTAGE-DEPENDENT EFFECTS OF AMLODIPINE IN SUBCUTANEOUS RESISTANCE ARTERIES ISOLATED FROM MAN

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Electrophysiological and ligand binding studies have indicated that the interaction of dihydropyridines with voltage-operated calcium channels may be influenced by the cell membrane potential. Amlodipine (A) is a novel dihydropyridine derivative which possesses calcium antagonist properties (Burges et al., 1987). In these studies we have examined whether A-induced inhibition of contraction of human resistance arteries is affected by the membrane potential.

Human resistance arteries were isolated from subcutaneous tissue removed during surgery and studied using a myograph (Mulvany & Halpern 1977). The kinetics of A-induced inhibition was studied under normal conditions in physiological saline (PSS). The rate of onset of A-induced inhibition over a 3h period was measured by examining the effect of A (100nM - 10μM) on contractions induced by 2min exposure to PSS in which 80mM KCl was substituted for 80mM NaCl ($KPSS_{80}$). The effect of amlodipine was expressed as % reduction of the control responses to $KPSS_{80}$ prior to addition of A. Offset of A-induced inhibition was studied in a similar manner by observing the recovery of response to $KPSS_{80}$ over a 5h period following washout of A (1μM). These results were compared with those obtained under depolarized conditions: for these studies tissues were depolarized by exposure to calcium free PSS containing 40mM KCl substituted for NaCl ($KPSS_{40}$). The rate of onset and recovery from A-induced inhibition were studied by repeated exposure to $KPSS_{80}$ as described above. Data derived from onset and offset studies were fitted to a single exponential function by non-linear regression analysis. Data are expressed as means ± s.e.means of n experiments.

Under normal conditions A caused a concentration-dependent inhibition of contraction induced by $KPSS_{80}$ (EC_{50} = 326nM, E_{max} = 94±2%, n=12). The rate of onset of A-induced inhibition was slow, the time constants for inhibition (τ) being 73, 27 and 7 min at 100nM, 1μM and 10μM respectively. Offset of inhibition under these conditions was also slow (dissociation rate constant (k_{off} = 0.0095 min). From these data the association rate constant (k_{on}) for A was calculated to be 27500 $M^{-1}min^{-1}$ and the calculated dissociation constant (K_d) was 345nM. Under depolarized conditions the effect of A was also concentration-dependent but A was considerably more potent (EC_{50} = 3nM, E_{max} = 95±3%, n = 21). In $KPSS_{40}$ the onset time constants for A-induced inhibition were 250, 59 and 33min at 1nM, 10nM and 100nM respectively and the k_{off} was 0.0003 min^{-1} giving an estimated k_{on} = 1887567 $M^{-1}min^{-1}$ and K_d = 0.2nM.

The interaction of amlodipine with the voltage-operated calcium channel of human isolated resistance arteries is extremely sensitive to the cell membrane potential. Depolarization by $KPSS_{40}$ caused a marked increase in potency and also increased the rate of association of the drug. These properties of A may be an important factor in explaining the long duration of action of this drug *in vivo*.

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429P VASOMOTOR EFFECT OF VASOACTIVE INTESTINAL PEPTIDE (VIP) AND [^{125}I]-VIP BINDING IN NORMAL AND DISEASED HUMAN CORONARY ARTERY

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The vasodilatory effect of vasoactive intestinal peptide (VIP) has previously been demonstrated in human coronary arteries. In this study we have examined the effect of tissue pathology on the response to VIP in human epicardial coronary arteries and used slide-mounted sections to establish altered [^{125}I] VIP binding to vessels. 26 vessel segments were utilized from 12 patients undergoing heart transplantation for organ bath studies.

The distribution and density of [^{125}I] VIP binding sites were established using in vitro autoradiography. Competition studies were carried out where sections were incubated in [^{125}I] VIP in the presence of increasing concentrations of unlabelled peptide. The degree of [^{125}I] VIP bound to vessel sections was determined both in a gamma counter and by densitometric analysis (Barnes et al, 1986).

VIP (10^{-12} to 10^{-7}M) elicited concentration-dependent relaxations of coronary artery segments which were pre-constricted with thromboxane-mimetic U46619 (20nM). The relaxations produced at 10^{-8} and 10^{-7} M VIP were equivalent to 37.3±8.6% and 69.3±5.8% (mean±s.e.mean) of the maximum dilatation induced by lug/ml glyceryl trinitrate in 'normal' arteries. This response (at 10^{-7}M of VIP) was significantly reduced to 39.3±6.8% of glyceryl trinitrate induced relaxation in diseased arteries ($p<0.005$).

There was concentration-dependent competition of [^{125}I] VIP binding by unlabelled VIP (10^{-10} to 10^{-6}M) in both 'normal' and diseased coronary arteries. Total binding was significantly reduced in diseased vessels (144± 39 amol mg^{-1}wet weight), compared to 'normal' vessels (448±177 amol mg^{-1}wet weight, $p<0.025$). Microscopic examination indicated that binding was associated with smooth muscle and perivascular regions, yet there was no evidence that [^{125}I] VIP bound to the endothelium.

The results highlight the role of vasoactive peptides in the control of blood vessel tone. The decreased responsiveness and reduced receptor binding of VIP in diseased coronary artery suggest a loss of peptidergic influences on vascular tone in atherosclerosis.

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430P CYCLOSPORIN A (CsA)-INDUCED CALCIFICATION: EFFECT OF VITAMIN D AND CALCITONIN (CT)

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Severe myocardial calcification accompanied by hypocalcaemia and hypomagnesaemia has been observed in CBA/Ca mice treated with CsA (Borland et al, 1985a & b). The work here investigates the effect of the calcium regulators, vitamin D and calcitonin, on CsA-induced cardiac calcification in CBA/Ca mice.
CBA/Ca mice (♂+♀ 20-25g) were obtained from Bantin and Kingman ltd., Humberside. All mice received food (R.M.B. diet, Heygate and sons, Northampton) and water ad lib. CsA (a gift from Sandoz) 80mg/kg and vitamin D (cholecalciferol, Sigma) 0.5mg/kg were dissolved in olive oil and administered orally in a volume of 0.1ml. Calcitonin (as calsynar, a gift from Rorer Pharmaceuticals) 10units/kg was diluted and neutralised with 0.15M phosphate buffer and administered by subcutaneous injection in 0.1ml. Groups of mice were treated daily for 14 days according to the treatments in the table.
Mice were killed 24 hours after completion of treatment. The hearts were removed for measurement of tissue calcium content and were dried to constant weight followed by digestion in nitric acid. Tissue calcium and plasma calcium and magnesium content were determined by atomic absorption spectroscopy and were compared using students t-test.

	Expt. No.	Olive Oil	CsA	Vit D	CsA + Vit D	PO_4 Buffer	Calcitonin	CsA + CT
Cardiac Ca^{++} μmol g^{-1} dry tissue	1)	8.2 ± 3.1	590.1 ± 108.4***	5.7 ± .3	903.0 ± 187.7	----------	----------	----------
	2)	4.2 ± 0.5	326.5 ± 77.3**	----------	----------	3.6 ± 0.2	2.7 ± 0.1	496.1 ± 37.5†
	3)	5.1 ± 0.6	50.7 ± 8.0***	6.2 ± 0.7	218.3 ± 43.5††	4.5 ± 0.4	6.9 ± 0.9	124.5 ± 28.5†
Plasma Mg^{++} mmol l^{-1}	1)	1.11 ± 0.04	0.82 ± 0.04***	1.20 ± 0.04	0.75 ± 0.05	----------	----------	----------
	2)	1.14 ± 0.03	0.74 ± 0.04***	----------	----------	1.31 ± 0.04	1.27 ± 0.02	1.00 ± 0.06
	3)	1.42 ± 0.06	0.80 ± 0.03***	1.43 ± 0.08	0.89 ± 0.06	1.36 ± 0.04	1.49 ± 0.09	0.96 ± 0.07

Values are mean ± S.E. n=5 * Significance of difference from control (*) $p<0.05$ (**) $p<0.01$ (***) $p<0.001$
† Significance of difference from CsA (†) $p<0.05$ (††) $p<0.01$ (†††) $p<0.001$

Plasma magnesium concentration was significantly reduced in all of the groups treated with CsA. Vitamin D at the dose employed had no significant effect on plasma calcium, however,calcitonin produced a transient fall within an hour of injection, but by 3 hours the plasma concentration was restored.
Cardiac calcification was visible macroscopically in all mice treated with CsA and the tissue calcium content reflected the observed macroscopic calcification, but hearts from male mice showed a greater calcification than hearts from female mice. There was no difference between the cardiac calcium content or plasma calcium concentration of vitamin D or calcitonin treated mice and their respective controls, but addition of vitamin D or calcitonin to CsA significantly increased the degree of cardiac calcification with no obvious effect on plasma calcium.
In conclusion CsA causes an increase in cardiac calcium content in CBA/Ca mice and this effect is magnified by doses of both vitamin D and calcitonin which are insufficient to produce prolonged changes in the plasma calcium concentration.

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431P EFFECTS OF DILTIAZEM ON REPERFUSION ARRHYTHMIAS IN HYPERTROPHIED MYOCARDIUM

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Hypertensive left ventricular hypertrophy is associated with ventricular rhythm disturbances and increases the risk of sudden cardiac death (McLenachan and Dargie, 1990). This study assessed the protective action of the calcium antagonist diltiazem against reperfusion arrhythmias in hypertrophied rat hearts *in vitro*.

Hearts were removed from normotensive and renovascular hypertensive Sprague-Dawley rats. They were Langendorff-perfused at 37°C with Krebs-Henseleit buffer, modified to contain K^+ 5.3 mM and Ca^{2+} 1.4 mM. After 10 min control perfusion, the left coronary artery was occluded close to its origin for 10 min, followed by 10 min reperfusion. Hearts were assigned to the following groups: normotensive control (NC, n=11); normotensive diltiazem-treated (ND, n=9); hypertrophied control (HC, n=9); hypertrophied diltiazem-treated (HD, n=9). Diltiazem 10 μg (or vehicle) was infused as a bolus into the aortic cannula 2 min before coronary artery occlusion. Cardiac electrograms recorded in the reperfusion period were analysed for incidence and duration of ventricular tachycardia (VT) and fibrillation (VF), following the diagnostic criteria advocated in the Lambeth conventions (Walker et al, 1988). Left ventricle masses and changes in heart rate were compared using Student's t-test; arrhythmia incidences were compared using the chi-square test with Yates' correction, and median arrhythmia durations were compared with the Wilcoxon test.

Relative left ventricle mass (g wet weight per 100 g body weight, quoted as mean ± s.e.mean) was significantly greater in hypertensive rats (HC 0.304 ± 0.008; HD 0.293 ± 0.008) than in normotensives (NC 0.221 ± 0.005; ND 0.220 ± 0.004) (HC v NC, $p < 0.01$; HD v ND, $p<0.01$). The decrease in heart rate during ischaemia (expressed as a percentage of initial rate during control perfusion period) was not affected by diltiazem (NC 15.6 ± 2.3; ND 17.3 ± 3.0; HC 18.5 ± 2.5; HD 13.1 ± 2.6; differences not significant). VT incidence was reduced from 82% in NC to 0% in ND ($p<0.01$) and from 100% in HC to 33% in HD ($p<0.01$). Median duration of VT was 23.9 s in NC and 17.4 s in HC (difference not significant). The calculated median duration of VT in HD was 0 s ($p<0.05$ compared with HC). VF incidence was reduced from 73% in NC to 0% in ND ($p<0.01$) and from 100% in HC to 22% in HD ($p<0.01$). Median duration of VF was 8.0 s in NC and 77.2 s in HC ($p<0.02$). Although VF occurred in 2 hearts in HD, as with VT in this group, the periods were so short that the calculated median duration for the group was 0 s ($p<0.01$ compared with HC).

In conclusion, the incidences of reperfusion-induced VT and VF were not significantly different in control hypertrophied and normotensive hearts . However, VF was of greater duration in hypertrophied hearts. Diltiazem administered before the onset of ischaemia, at a dose which was not negatively chronotropic, completely abolished reperfusion ventricular arrhythmias in normal hearts and attenuated their incidence and duration in hypertrophied hearts.

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432P EFFECTS OF LOCAL OR INTRAVENOUS ADENOSINE ON TISSUE INJURY IN A CANINE MODEL OF REGIONAL MYOCARDIAL ISCHAEMIA AND REPERFUSION

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Experimental evidence suggests that administration of adenosine may reduce infarct size (Olafsson *et al.*,1987) and decrease the incidence of ventricular arrhythmias in anaesthetised dogs (Wainwright & Parratt, 1989). We have now measured the effects of exogenous adenosine in a canine model of regional myocardial ischaemia (ISC) and reperfusion (REP). Under pentobarbitone/chloralose anaesthesia, animals (13.8±0.9 kg) were subjected to 90 min left anterior descending coronary artery (LAD) occlusion to initiate ISC, followed by 120 min REP. In Group 1 (n=4) saline was given i.v. at 1 $ml.min^{-1}$ for 60 min starting at 75 min ISC. Animals in Groups 2 (n=5) and 3 (n=4) received 16$mg.kg^{-1}$ adenosine at 3.75$mg.min^{-1}$ i.v. and directly into the LAD respectively (this dose has been previously shown to produce systemic vascular effects, Olafsson *et al.*, 1987). Regional myocardial blood flow (RMBF) was determined serially with radiolabelled microspheres and ventricular function (segment shortening , SS) was assessed by sonomicrometry. At the end of REP, dual dye perfusion of excised hearts with Evans blue and tetrazolium chloride was used to assess area at risk (R) and infarct size (I) respectively. I and R were expressed as a % of the left ventricle. An inverse relationship between collateral flow and I/R ratio was observed ($I/R = 131.3 - (32.2\ \ln Q)$, $r^2 = 0.88$) in all groups. I/R ratios were not significantly different between groups (36±12%, 33±11%, 24±15%). RMBF in early REP (expressed as % flow to the non-ischaemic region) was not significantly altered between groups in the post-ischaemic subepicardium (171±30%, 141±28%, 213±91%) or subendocardium (154±27%, 106±35%, 146±49%). No significant difference was observed between groups in ischaemic region SS at 90 min ISC (-5.4±3.5%, -3.2±1.3%, -0.7±1.4%), 5 min REP (5.7±6.5%, 5.9±0.7%, 2.8±4.5%) or 120 min REP (0.0±5.4%, -0.1±1.2%, -6.4±1.8%). The incidence of ventricular arrhythmias was similar between groups during ISC and REP (Table 1).

Table 1 Number of ventricular premature beats (VPBs) ±s.e.mean, and incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF)

Group	ISCHAEMIA			REPERFUSION		
	VPBs	VT	VF	VPBs	VT	VF
1 (n=4)	70±18	0/4	0/4	6071±2387	2/4	0/4
2 (n=5)	75±26	0/5	0/5	3762±1366	1/5	0/5
3 (n=4)	208±125	1/4	0/4	1436± 999	2/4	1/4

The results from this study do not indicate an action for adenosine in either limiting infarct size or as an anti-arrhythmic agent at the dose used in this study.

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433P EFFECTS OF OUABAIN AND CLOFILIUM ON BRETYLIUM UPTAKE IN GUINEA-PIG HEART VENTRICULAR TISSUE *IN VITRO*

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Bretylium and clofilium are effective, structurally related class III antiarrhythmic/antifibrillatory agents extensively accumulated in myocardial tissue, with their actions apparently correlating better with myocardial rather than plasma concentrations (Namm *et al.*, 1975; Anderson *et al.*, 1980; Lindstrom *et al.*, 1982). In the case of bretylium, high concentrations were found in the Na,K-ATPase rich subcellular fractions of cardiac tissue from normal and immunosympathectomized animals (Namm *et al.*, 1975), indicating that neuronal uptake was unlikely to account for the accumulation. In the present work, the effects of ouabain and clofilium were observed on bretylium uptake in myocardial tissue to see if there is evidence of Na,K-ATPase being involved in bretylium and clofilium accumulation.

[^{14}C]Bretylium iodide was synthesized from N-*o*-bromobenzyl-N-ethyl-N-methylamine and [^{14}C]methyl iodide (Boura *et al.*, 1960) and uptake determined in guinea-pig heart left ventricle slices (**A**) after Na^+-loading to stimulate Na/K pump activity. Slices (20-50mg & 0.5mm thick) were incubated with [^{14}C]bretylium in 2ml Krebs-Henseleit solution gassed with 5% CO_2/95% O_2, for 30min at 37°C and the results corrected for extracellular drug present. In Langendorff-perfused hearts (**B**), [^{14}C]bretylium uptake was measured in left ventricle slices after perfusion. Recorded cardiac contractions were negligibly affected by the concentrations of test drugs used.

Bretylium uptake was hyperbolic (K_m 2.8μM) at low concentrations (1-30μM applied bretylium) in both **A** and **B**, with maximal tissue/medium concentration ratios (6 and 10.5 respectively) observed at 1μM and the uptake becoming linear above 30μM. In **A**, ouabain (1mM) had marked effects in the hyperbolic region (79% inhibition at 1μM decreasing to 59% at 10μM and 29% at 30μM) and minimal effects in the linear region. In **B**, 1μM bretylium uptake was significantly ($P < 0.05$) inhibited (21.2%) by 3μM ouabain and (52.6%) by 10μM clofilium. Clofilium (0.01-1mM) competitively inhibited bretylium (1 & 10μM) uptake in **A**, with little evidence of any effect when ouabain (1mM) was present, indicating that ouabain-sensitive uptake was principally inhibited. After efflux, the percentages lost and retained in the tissue corresponded closely to the ouabain-insensitive and ouabain-sensitive components respectively, suggesting that the latter may represent a relatively non-labile pool of drug within the tissue.

The results demonstrated that Na,K-ATPase was closely involved in the uptake and possibly in the tissue retention of bretylium, with this being most evident in the 1-10μM range, i.e. approximating to the plasma concentrations achieved in antiarrhythmic therapy (Rapeport, 1985). The competitive inhibition of bretylium uptake produced by clofilium suggested that both drugs may be taken up by a similar mechanism. It also appears that other quaternary ammonium compounds, e.g. TEA and certain bisquaternaries, may be taken up by Na,K-ATPase-mediated systems in other tissues (Holm, 1976; Meijer *et al.*, 1971).

N-o-bromobenzyl-N-ethyl-N-methylamine and clofilium were gifts from Wellcome Research Laboratories and Eli Lilly Inc. respectively.

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434P ISRADIPINE DYNAMICS AND KINETICS IN THE ISOLATED RABBIT HEART

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The cardiac effects of increasing concentrations of the newer dihydropyridine Ca^{++}-channel blocker isradipine from 1.64 pM to 232 nM were studied in isolated spontaneously beating rabbit hearts. Inhibitory responses with regard to contraction amplitude, contraction velocity and oxygen consumption were biphasic. Inhibitory E_{max}-values (derived by nonlinear regression analysis) of the second phase were 104, 103 and 87 % (IC_{50}: 7.1, 6.3 and 28.7 nM), respectively, whereas those of the initial phase were 24.7, 25.9 and 19.5 % (IC_{50}: 0.012, 0.038 and 0.026 nM). 'Hill' slope coefficients were about 0.6- 0.7 for both phases. These findings seem to indicate two sites of myocardial action. A monophasic inhibition of frequency reached a maximum of only 21 %. Myocardial efficiency decreased rapidly above 1.8 nM isradipine which also was the concentration border between the bihasic responses.

The ECG-derived PQ-interval showed a rapid increase (maximum 46 %) at drug concentrations above 1 nM. Complete AV-block and ventricular asystolia occurred in half of the hearts at the second highest (99 nM) and in all except one at the highest concentration. SA-node activity was retained in 9 of 10 hearts at the second highest and in 3 at the highest drug exposure. The QRS-interval and the frequence-corrected QT-interval did not increase significantly. Coronary flow-rate showed no initial increase, but a decrease to 70 % of control at the highest concentration. Supplementary in-vitro studies on rabbit coronary artery ring-preparations contracted with 124 mM K^+ showed, however, a relaxant E_{max}-value for isradipine of about 100 % and an inhibitory EC_{50}-value of 0.63 nM with a 'Hill' slope coefficient of 1.1.

At toxic concentrations isradipine showed a kinetic monophasic accumulation in the rabbit heart of about 44-fold with a half-time of 10.6 min. The myocardial disposition of the drug was three-phasic with half-times of 4.3, 13.8 and 75 min., respectively. These findings seem in agreement with an observed biphasic and only partial reversibility of the dynamic effects within 30 min. after stopping drug exposure.

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435P RELATIONSHIPS BETWEEN LIPOPHILICITY, BINDING AND VOLUME OF DISTRIBUTION FOR SOME PHENOTHIAZINE DRUGS

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Phenothiazines are generally highly bound to plasma proteins (>70%) and red blood cells; this influences their distribution and pharmacokinetics. Hence binding and its relationship to various physicochemical and pharmacokinetic parameters are important in drug design. Plasma and blood cell binding were determined for trimeprazine and 14 other closely related phenothiazines. The unbound fraction (fu) in fresh rabbit plasma was measured by equilibrium dialysis. Blood cell affinity (Kbc/pw) was derived using fresh rabbit blood. Log P (octanol/water) values were calculated from experimentally determined pK_a and log D values. Plasma and buffer samples were assayed using HPLC methods.

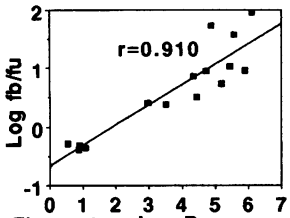

Figure 1

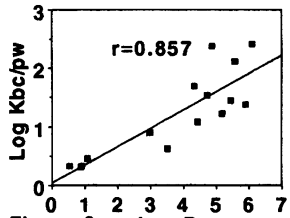

Figure 2

Figures 1 and 2 show that significant correlations exist between plasma binding affinity [fraction bound (fb) /fu] and log P and between blood cell affinity (Kbc/pw) and log P. These findings indicate that lipophilicity increases the binding to plasma proteins and binding/partitioning to blood cells. The cluster of compounds with lower log P values are all sulphoxides, associated with plasma binding of approximately 30%. Their exclusion from the analyses still results in significant, albeit lower, correlations.

These results are in concordance with the work of Krieglstein *et al.* (1972) who found a positive correlation between serum albumin binding and log P of a group of phenothiazines, but disagree with Schley & Muller-Oerlinghausen (1986) who found no correlation between binding to α_1-acid glycoprotein and log P for a group of phenothiazines/tricyclics.

Steady-state volume of distribution (Vss) was determined for 6 of the compounds in 5 male New Zealand White rabbits. Vss was found to correlate significantly with both fu ($r = -0.928$; $p < 0.01$) and log Kbc/pw ($r = 0.814$). The negative correlation between Vss and fu suggests that tissue/plasma binding ratios increase with increasing plasma binding (Gibaldi & McNamara, 1978). Hence increasing lipophilicity within this congeneric series, not only increases plasma binding (fu vs. log P gave $r = -0.958$ for these 6 compounds) but produces an overwhelming increase in tissue binding, resulting in a higher Vss.

We conclude that the binding of some drugs, to plasma proteins, blood cells and tissues can be related to lipophilicity. Hence simple physicochemical and *in vitro* measurements may be useful for pharmacokinetic predictions in the design of new drugs.

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436P THE PHARMACOLOGICAL ACTIVITY OF A TOPICALLY APPLIED 5% IBUPROFEN CREAM

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A frequently reported side-effect of orally administered non-steroidal anti-inflammatory drugs (NSAIDs) is gastro-toxicity. Topical NSAIDs are expected to minimise gastro-toxicity by localising the drug, with insignificant amounts reaching the plasma (Novack et al., 1987). Recent reports however, suggest topical anti-inflammatory activity may in part be systemically mediated. (Nolan et al., 1988, Elliott & Rostron, 1988, Radermacher et al., 1991).

Carrageenan paw oedema model (Winter et al., 1962) was used to assess the pharmacological activity of both oral (30mg/kg) and topical (300mg) ibuprofen doses, administered 30 minutes prior to 0.1ml injection of 2% carrageenan solution into the right hind paw of male Wistar rats. Topical application was to the right (ipsilateral) or left (contralateral) hind paw. Paw volumes were measured by displacement using a mercury plethysmograph, and percentage inhibition of oedema determined by comparison with untreated control groups. Plasma and tissue drug levels were determined using a modified HPLC method (Skellern & Salole, 1975).

Oral administration produces maximum inhibition of oedema at 3 h when plasma levels are also at a maximum (Table 1). In comparison topical administration produces high tissue levels but significantly lower plasma levels ($P<0.05$). The inhibition of oedema however is lower than that achieved with oral administration, suggesting tissue drug levels are not critical in producing an anti-inflammatory effect. Contralateral application produces high drug levels in the inflamed paw, at 3 and 4 h these are over 50% greater than achieved orally. Anti-inflammatory activity however, is inferior and only when plasma levels reach a maximum at 4 h is the reduction in oedema significant. These results suggest topically applied ibuprofen cream is transported into the circulation, hence facilitating a systemically mediated anti-inflammatory effect, and thus supporting previous findings.

Table 1. The plasma and inflamed paw levels and anti-inflammatory effect of ibuprofen

Route	Oral		Ipsilateral		Contralateral	
Time (h)	3	4	3	4	3	4
Plasma level ($\mu g\ mL^{-1}$)	6.04±0.65	2.08±1.06	2.00±0.37	1.92±0.67	1.50±0.37	1.89±0.55
Paw level ($\mu g\ mL^{-1}$)	3.40±0.85	4.24±1.03	13.50±3.14	8.24±2.69	8.24±2.76	7.66±2.62
Inhibition of oedema (%)	53.30±9.53**	38.00±17.34*	41.41±10.36**	28.60±6.02	28.70±5.51	35.60±8.44*

*P<0.05 **P<0.005 (n)=10

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437P DIURNAL PLASMA NICOTINE AND COTININE LEVELS AND TOLERANCE TO NICOTINE IN MICE AFTER 7-WEEK ORAL NICOTINE ADMINISTRATION

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The relevance of oral administration of nicotine for studying the effects of long-term nicotine exposure in mice was investigated. Mice received nicotine in the drinking water as sole fluid for 7 weeks. Six weeks old male NMRI mice were divided into two equal groups of equivalent body weight (nicotine group 28±0.9 g; control group 27±0.8 g, mean±s.e.;n=200). They had free access to mouse chow. Lights were on from 06 h 00 min to 18 h 00 min. The initial concentration of nicotine in the drinking water was 50 μg/ml, and it was increased in amounts of 50 μg/ml at 3-4 day intervals to 300 μg/ml during 3 weeks and after that at 7 day intervals to 500 μg/ml which concentration was offered for the last 7 days. Body weight and fluid intake were recorded once a week. After 7 weeks part of the mice were killed by decapitation at 05 h, 11 h, 15 h or 21 h. The concentrations of nicotine and its main metabolite cotinine in the plasma were determined as previously described (Leikola-Pelho *et al.*,1990). The rest of the mice were withdrawn for 24 h and challenged with 1 mg/kg of nicotine s.c. The rectal temperatures were measured at 15, 30, 60 and 90 min.

Nicotine could be administered up to concentration of 350 μg/ml without any significant effect on fluid intake or weight gain. After that the fluid intake and weight gain started to decrease so that the mice treated with 500 μg/ml nicotine drank 43% less and weighed 9% less than the controls. The largest plasma concentration of nicotine, 155±30 ng/ml was found at 05 h, and that of cotinine, 4697±328 ng/ml at 11 h (mean±s.e.;n=9). The smallest values of nicotine, 38±13 ng/ml, and cotinine, 1280±478 ng/ml, occurred at 21 h. Thus the diurnal drinking pattern of the chronically nicotine-treated mice was typical for nocturnal animals. The nicotine challenge dose of 1 mg/kg s.c. decreased the rectal temperature of control mice at 15 min by 3.7±0.2°C and at 30 min by 4.0±0.4°C. In the withdrawn mice the corresponding decreases were at 15 min 3.0±0.2°C ($P<0.05$) and at 30 min 2.5±0.3°C ($P<0.05$).

These results agree with previous finding that chronic treatment of rodents with nicotine results in the development of tolerance to the effects of an acute challenge dose of the drug on the body temperature (Marks *et al.*,1986). Further our results show that nicotine can be administered chronically orally to mice in doses high enough to induce tolerance.

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438P BLOOD ALCOHOL CONCENTRATIONS AFTER LOW-ALCOHOL BEER

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In recent years low alcohol beers have become available. In this study we investigated the rate of absorption of alcohol from low alcohol beer (1% v/v).

Experiment 1 - Comparison of low alcohol beer with regular beer (4%)

Male volunteers were randomly assigned to three groups and drank beer over 1 hr 50 min. Those in Group 1 consumed 4 pints of 1% beer (Tennent's LA); Group 2 consumed 6 pints of 1% beer; Group 3 consumed 1½ pints of 4% beer (Carling Black Label). The total alcohol consumed was 18.2 g for Group 1 and 27.25 g for Groups 2 and 3. Blood alcohol concentrations (BACs) were determined from breath at 60 and 120 min. If the 120 min value was noticeably higher than the 60 min value, another reading was taken at 150 min to confirm that the 120 min value was a peak.

Experiment 2 - "Topping up"; 4% beer plus 1% beer

Male volunteers were randomly assigned to two groups and drank beer over 1 hr 50 min. They drank either 3 pints of 4% beer followed by 2 pints of 1% beer or 3 pints of 4% beer followed by ½ pint of 4% beer. In each case, the total alcohol consumed was the same (63.6 g).

Treatment	**Dose**	**Mean BAC (120 min) ± s.e.m. in mg/100 ml blood**	**n**
4 pints of 1% beer	18.2 g	7.3 ± 1.23[a]	7
6 pints of 1% beer	27.25 g	12.3 ± 1.91[b]	6*
1½ pints of 4% beer	27.25 g	31.8 ± 3.10[c] ([a] vs [b] - n.s.; [b] vs [c] $p < 0.05$)	8
3 pints 4% beer then 2 pints 1% beer	63.6 g	83.4 ± 5.25	5
3 pints 4% beer then ½ pint 4% beer	63.6 g	104.0 ± 4.60 ($p < 0.001$)	5

*One subject found the volume excessive, "lost interest" and was withdrawn.

These results suggest that it is unlikely that a subject could exceed the 80 mg% BAC UK driving limit as a result of drinking 1% beer alone. Similarly, the BAC rises less than would be expected on the basis of alcohol intake when 1% beer is consumed after 4% beer.

439P IVERMECTIN INCREASES THE ANION CONDUCTANCE OF THE RESTING MEMBRANE OF *ASCARIS SUUM* MUSCLE BAG CELLS

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The anthelmintic ivermectin (IVM) activates anion channels in excised patches of *Ascaris* muscle bag cells (Martin & Pennington, 1989). At the whole cell level however, no conductance increase is seen (Holden-Dye & Walker, 1990).

In this study we exploited the phenomenon that increasing a particular ion conductance makes membrane potential more sensitive to changes in the extracellular concentration of that ion. Two sections were taken from a worm, one section was incubated for 1hr in artificial perienteric fluid (APF) + 10μM IVM in 1% DMSO, or APF + 1% DMSO. Single microelectrode intracellular recordings were made from cells in the unincubated sections. Typical resting membrane potential was -30mV. Exposing cells to NO_3^- caused a hyperpolarisation due to NO_3^- influx through resting anion channels.

Following incubation the same protocol was performed on the other section. APF expressed in mM contains NaCl (67), NaGluconate (67), TRIS (5), KCl (3), $MgCl_2$(15.7), EGTA (1). For the NO_3^- solution, NaCl and NaGluconate were replaced with $NaNO_3$. Cells incubated in APF + 1% DMSO exhibited a smaller hyperpolarisation than their corresponding unincubated controls. The hyperpolarisations being -9.0 ±0.96mV (n=11, 6 preps.) and -11.2 ±1.3mV (n=8, 6 preps.) respectively. Incubation with IVM increased the hyperpolarisation elicited by NO_3^-. This being -12.9 ±1.3mV (n=10, 6 preps.), compared to the unincubated -10.4 ±1mV (n=7, 6 preps, Figure 1). The differences between the two incubated groups are significant to P<1%.

The results suggest that IVM increases the anion conductance of *Ascaris* muscle bag cells.

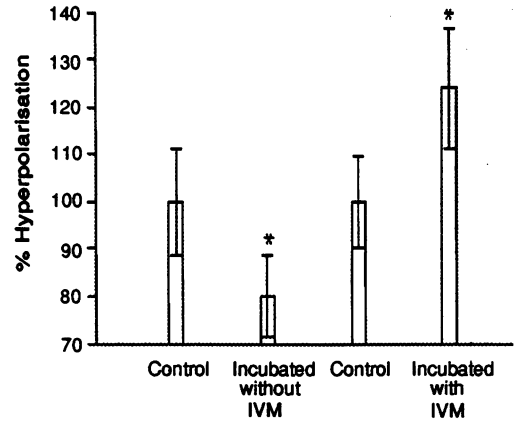

Figure 1. Data normalised with respect to the hyperpolarisation in the presence of NO_3^- before incubation. Asterisks indicate significant difference (p<1%) between incubated groups.

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440P FOOTPRINTING STUDIES ON THE INTERACTION OF BLEOMYCIN WITH DNA: ROLE OF THE METAL ION

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The bleomycins are a family of structurally related glycopeptide antibiotics which are used in cancer chemotherapy. These agents act by introducing single-stranded breaks into DNA in a reaction that requires stoichiometric amounts of divalent metal ions and molecular oxygen (Burger *et al.*,1981). Bleomycin can form complexes with several transition metals, the biologically relevant complex being proposed to be with iron. We have previously shown that in the presence of cobalt bleomycin binds to DNA but does not induce DNA breaks (Fox *et al.*, 1987). In this study we further examine the ability of various metal-bleomycin complexes to bind to DNA, by a variety of footprinting techniques.

DNaseI footprinting studies at 37°C using various metal-bleomycin complexes revealed that Co(II) was the only ion capable of yielding specific footprints. Experiments performed at lower temperatures (4°C) or with probes more sensitive to weaker drug binding revealed some selective binding of the Mn(II) complex. The importance of the nature of the metal ion was confirmed by reaction with diethylpyrocarbonate and potassium permanganate, probing DNA structural changes induced by the antibiotic. The Co(II) complex increased the reactivity of A and T residues adjacent to bleomycin cleavage sites, whereas other metal ions had no effect. The metal free drug (i.e. in the presence of an excess of EDTA) had no effect on DNA cleavage by these agents. Gel retardation studies, examining the effect of the drug on the mobility of supercoiled and linear DNA fragments in agarose gels, revealed that only the Co(II) complex effected any significant change in mobility.

We conclude that the divalent metal ion has profound affects on the binding of bleomycin to DNA. Only the Co(II) complex is sufficiently stable to be detected by footprinting techniques.

KPN is a CRC research student. KRF is a Lister Institute Research Fellow.

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441P THE ANTITUMOUR AGENT ECHINOMYCIN ALTERS LOCAL DNA STRUCTURE

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Footprinting studies have previously shown that the antitumour antibiotic echinomycin is selective for the sequence CpG and that enhancements in nuclease susceptibility are often detected adjacent to drug footprints (Low *et al.*, 1984). The enhancements have been explained by suggesting that the antibiotic induces DNA structural changes which are propagated along the DNA helix. In this study we further examine these structural changes by performing DNaseI footprinting studies on synthetic DNA fragments containing the sequences $A_{15}CGT_{15}$, $T_{15}CGA_{15}$, $T(AT)_8CG(AT)_{15}$ and $(TAA)_4CG(TTA)_4$. For each fragment we observe footprints around the central CpG though these changes are much clearer when the echinomycin binding site is surrounded by alternating rather than homopolymeric regions of A and T.

Regions of homopolymeric A and T are generally cut very poorly by DNaseI on account of their narrow minor groove. In the presence of echinomycin dramatic increases in cleavage of the T stretches are observed for both $T_{15}CGA_{15}$ and $A_{15}CGT_{15}$. Cleavage of the runs of A is enhanced closest to the drug binding site for $T_{15}CGA_{15}$, but fades out towards the 3'-end. In contrast with $A_{15}CGT_{15}$ the As are only enhanced towards the 5'-end, furthest from the drug binding site, possibly due to secondary drug binding at the CpA step at the edge of the insert. Footprinting studies at lower temperature (4°C) failed to show either footprints or enhancements , suggesting that the two are related events and that the structural changes are a necessary consequence of drug binding.

DNaseI cleavage of alternating AT in the absence of the antibiotic reveals a pattern in which ApT is cut much better than TpA. In the presence of low concentrations of drug cleavage of TpA is much enhanced. With higher concentrations of echinomycin DNaseI cleavage of $(AT)_n$ reveals an unusual pattern with a four base pair repeat. We explain this by invoking secondary binding of the ligand within the AT region. We examined this unusual pattern in more detail by using a fragment containing the sequence $(TAA)_4CG(TTA)_4$. In this case the cleavage pattern in the AT-region is not affected by the antibiotic, though there is a general increase in cutting efficiency. We conclude that echinomycin induces changes in regions flanking its binding sites, which vary according to the nature of the surrounding sequences.

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442P THE EFFECTS OF MONOCLONAL ANTIBODIES TO TUMOUR NECROSIS FACTOR IN ANIMAL MODELS OF CHRONIC ARTHRITIS

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Indirect evidence has implicated soluble cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF) in the pathology of rheumatoid arthritis . We have now used neutralising antibodies to TNF to define the importance of this cytokine in the induction of experimental arthritis in the rat and rabbit.

Collagen-induced arthritis (CIA) was induced in 18 Wistar rats by the intradermal injection of soluble bovine type II collagen in Freund's incomplete adjuvant (Staines et al 1981). One group of 9 animals received intraperitoneal injections of the Hamster anti-murine/rat TNF monoclonal antibody (mAb) TN3 19.12 (2.5mg/animal/dose) at day -1 and at 1,3,6,8,10,13,15 and 17 days following immunization. Control animals (9) received an equal volume of vehicle. Signs of disease were scored visually daily on a blind basis and sera was collected at various intervals for measurement of anti-collagen antibody titres. Animals were killed at day 22 and the feet removed for histological analysis. Antigen-induced arthritis in the rabbit was induced by immunization with ovalbumin followed by intraarticular injection of this protein into one knee, the contralateral joint receiving vehicle (Henderson & Glynn 1981). One group of 4 rabbits received murine anti-rabbit TNF mAb R2 (1.5mg/kg:im) 2 hours prior to induction of arthritis and a second dose 26 hours later. Animals were monitored for joint swelling and were killed at 72 hours. The proteoglycan content of the cartilage and of the synovial fluid was measured as described (Pettipher et al 1986).

After 14 days all the control rats showed visible signs of arthritis and at 22 days histological examination revealed extensive inflammatory damage. In the group receiving anti-TNF mAb, arthritis was delayed to 17 days and histology showed that 2 out of 8 animals had virtually no inflammatory damage.

The anti-TNF mAb had no influence on joint swelling in rabbits but significantly inhibited the loss of proteoglycan from articular cartilage. 3 days after induction of arthritis the inflamed joints of control rabbits contained 71.3±14.8% (mean±SEM) of the proteoglycan content of the control joints compared to 95±11.2% in the inflamed joints of anti-TNF-treated rabbits. This difference was confirmed when the proteoglycan content of the synovial fluid was measured. The anti-TNF therapy halved the content of proteoglycan in inflamed synovial fluid from 45.3±6.7ug/ml to 24.7+-9.3ug/ml ($p<0.5$).

These preliminary experiments show that neutralization of TNF can influence the induction of two forms of experimental arthritis and suggests a role for TNF in the induction of chronic destructive arthritis.

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443P RELATIONSHIP OF FEEDING, PLASMA AND GASTRIC MUCOSAL INDOMETHACIN CONCENTRATIONS AND PGE_2 SYNTHESIS WITH INDOMETHACIN-INDUCED GASTRIC LESIONS IN THE RAT

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The occurrence and severity of gastric ulceration induced by non-steroidal anti-inflammatory drugs (NSAID) is influenced by food intake which also has a complex influence on the bioavailability of NSAID. We have investigated the relationship between plasma and gastric mucosal indomethacin concentrations, gastric mucosal PGE_2 synthesis and gastric damage over a 5h period in rats given 150mg/kg indomethacin orally in 2.5% carboxymethylcellulose (CMC) or CMC alone. Prior to dosing one group of rats were fasted for 24h and one allowed free access to food (n = 7 to 9 per group). Blood samples were taken by cardiac puncture under ether anaesthesia at 0.5, 1, 2, 3 and 5h after indomethacin. The animals were then killed and the stomach removed and washed extensively with 3 lots of 20ml Krebs buffer at 1°C. Stomachs were photographed and mucosal discs prepared with a modification of the method of Mellarange & Rashbrook (1986). Discs were incubated for 15min at 37°C in Krebs buffer and PGE_2 synthesis measured by RIA. The remaining mucosa and plasma were stored at -20°C for indomethacin determination by HPLC using a modification of the method of Bernstein & Evans (1982). Ulcer areas were assessed by computer planimetry from photographic slides. PGE_2 synthesis (ng/mg protein ± sd) in control animals was 3.75 ± 2.86 (fasted) and 1.92 ± 0.70 (fed). Indomethacin significantly inhibited PGE_2 synthesis without discrimination in both feeding groups and at all time points by 84.4 ± 5.5%. In the fasted group, plasma indomethacin and lesion area increased progressively during the 5h period and both differed significantly from the fed group at 5h. The concentration of indomethacin in gastric mucosa was significantly greater in the fasted animals throughout the 5h.

Table 1 Indomethacin concentrations in plasma and gastric mucosa and gastric damage

Time (h)		0.5	1	2	3	5
Plasma indomethacin (µg/ml)	fasted	84.2 ±26.4	109.4 ±58.0	188.4 ±110.1	253.4 ± 89.1	348.1 ±53.8*
	fed	98.4 ±46.8	126.8 ±81.1	209.3 ± 70.1	218.7 ±106.3	233.5 ±63.4
Mucosa indomethacin (µg/mg dry weight)	fasted	0.71± 0.22**	0.88± 0.44**	0.69± 0.34**	0.51± 0.26**	0.48± 0.12**
	fed	0.19± 0.07	0.20± 0.08	0.20± 0.06	0.22± 0.09	0.16± 0.10
lesion area: % total stomach area	fasted	0.11± 0.10	0.26± 0.25	0.87± 1.41	1.85± 1.59	3.54± 2.49*
	fed	0.05± 0.08	0.14± 0.11	0.18± 0.19	0.38± 0.30	0.80± 0.59

* P<.05 **P<.002 Wilcoxon's rank sum test, compared with corresponding values in fed group (n = 7-9).

There was a linear relationship (r=0.98;P<0.001) between the cumulative exposure to indomethacin, as assessed by the area under the mucosal indomethacin concentration-time curve (AUC) and lesion score for fed and fasted groups combined. The plasma AUC values were also related linearly to lesion score but the fed (r=.99; P<0.001) and fasted groups (r=0.99; P<0.001) showed different slopes in their concentration effect relationships.

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444P CUTANEOUS RESPONSES TO POLY-L-LYSINE IN THE RABBIT

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Intradermal (i.d.) injection of cationic proteins (CP) extracted from human platelet granules (Nachman et al, 1972) or of polycations such as poly-L-lysine (PLL; Needham et al, 1988) produced plasma protein extravasation in rabbit skin. CP showed a biphasic permeability response whereas only the acute phase of the PLL response was studied. The oedema response to subplantar injection of poly-L-arginine, in the rat, was inhibited by local pretreatment with heparin (Antunes et al, 1990). We have, therefore, studied the time course of the permeability response to PLL and the effect of heparin on this response in rabbit skin.

Plasma protein extravasation (PPE) in the skin of male, New Zealand White rabbits (2.0-2.8 kg) was quantified using ^{125}I-albumin (Williams, 1979). Evans blue dye was used as a visual marker. There were 6 replicates of each treatment in each rabbit.

Extravasation was measured over a 1h period in sites injected i.d. 2,1 or 0h prior to intravenous (i.v.) injection of radiolabel. Substantial leakage of plasma protein was evident up to 2h following i.d. PLL (100ug). (2h-38.8±3.9µl; 1h-60.2±3.4µl; 0h-92.4±13.0µl; saline 0h-7.9±1.2µl; n=3). In contrast, sites injected with histamine plus PGE_1 (HA+PGE_1; 1ug+150ng) showed little extravasation beyond the first hour (1h-14.1±3.8µl; 0h-297.5±97.2µl; n=3). There was no significant accumulation of radiolabelled platelets in PLL sites showing substantial PPE (n=3; results not shown). It was noted that the PLL sites, viewed from the inner aspect of the skin, presented a mottled appearance compared to the uniform blueing at sites injected with HA+PGE_1.

Extravasation was measured over a 3h period in sites pretreated with saline (sal) or heparin (hep; 5U-100U) and superinjected 5 min later with PLL or HA+PGE_1. The PLL response was significantly inhibited by the highest dose of heparin (sal-136.7±16.7µl; hep 100U-46.2±7.9µl; n=3 $p<0.005$). In contrast, this dose of heparin tended to enhance responses to HA+PGE_1 (sal-105.5±6.3µl; hep 100U-169.8±26.4µl; n=3).

These results suggest that PLL produces PPE in rabbit skin by a mechanism distinct from that of a classical permeability mediator such as histamine.

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445P CROSS IMMUNOREACTIVITY BETWEEN CYTOCHROME P_{450} REDUCTASE AND NITRIC OXIDE SYNTHASE

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Recent work has demonstrated that there is a close similarity between some of the functional domains of nitric oxide synthase (NOS) and cytochrome P_{450} reductase (CPR) (Bredt et al., 1991). It has also been shown that both of these enzymes can carry out similar reactions with artificial electron acceptors such as nitroblue tetrazolium (NBT) (Hope et al., 1991).

In this study we have investigated whether these similarities extend to the ability of a polyclonal antibody raised to CPR in rabbits, to cross-react with NOS. The catalysis by NOS of the conversion of arginine to citrulline, generating NO in the reaction, was investigated by incubating a whole rat brain homogenate with [^{3}H]arginine, followed by ion exchange chromatography (Bredt & Snyder, 1991).The production of citrulline (and thus NO) was inhibited by the CPR antibody in a concentration-dependent manner. Cross immunoreactivity between the two species was also demonstrable upon western blot analysis. This cross immunoreactivity between CPR and NOS explains the co-localisation of CPR and diaphorase activity in rat brain neurones.

Neuronal NADPH diaphorase, a 150 kDa flavoprotein, exhibits considerable similarity to CPR (Kuonen et al., 1988; Kemp et al., 1988) and diaphorase histochemical staining of NOS-positive neurones has recently been shown to be due to the activity of NOS itself (Hope et al., 1991). CPR immunoreactivity has also been seen in many other neuronal types and it remains to be determined whether this is due to NOS or CPR. Rat brain does contain significant amounts of CPR as determined by the catabolic actions of the cyt P_{450} system. Colorimetric assays using NBT or other electron acceptors may however be ambiguous.

The finding of cross immunoreactivity reported here, coupled with the responsibility of NOS for diaphorase staining, provides little credence for the suggestion that selective sparing of diaphorase neurones in Huntington's disease, or when compromised by ischaemia or excitotoxic insult, could be due to a CPR detoxification mechanism.

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446P THE EFFECT OF SIMULTANEOUS EXPOSURE TO VARIOUS SOLVENTS ON THE (Ca^{2+}/Mg^{2+})ATPase ACTIVITY OF THE SYNAPTOSOMAL MEMBRANE

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It has been established that exposure to organic solvents affects both the enzymes and the lipid part of the nerve cell membrane, (Korpela & Täthi, 1987, Edelfors & Ravn-Jonsen, 1990). Since most of the industrial solvents are mixtures, research on solvent toxicity should include studies on the interaction between various solvents. Therefore, the in vitro effect of ethanol, n-butanol, n-hexanol, n-dodecanol and n-hexane on the nerve cell membrane was studied. Synaptosomal membranes were incubated simultaneously with two of the solvents in question. As an indicator of the (Ca^{2+}/Mg^{2+})ATPase activity, released inorganic phosphate was measured. Incubation with the alkanols affected the enzyme as shown in tabel I.

Table I: The effect of solvents on the synaptosomal ATPase activity in vitro.

	Ethanol(o/oo)		1-Butanol(sat.)		1-Hexanol(sat.)		1-Dodecanol(sat.)		n-Hexane(sat.)	
	2	4	1/16	1/8	1/8	1/4	1/8	1/4	1/8	1/4
Pure solv.	88±4*	85±4*	61±2*	39±4*	88±2*	70±3*	86±4*	83±5*	98±3	97±5
+ Ethanol (4o/oo)				25±3#				81±3		81±3
+ 1-Butanol (1/8 sat.)					3±3#			61±7#		50±8#
+ 1-Hexanol (1/8 sat.)								73±2		76±3

The values indicate % of activity in proportion to control ±s.d. (n) = 4 experiments in triple. Activity in control membranes: 10.9±0.37 nmol P_i/μg protein·hr. (100%±3)
*: Significant different from control. #: Significant different from the pure solvents.

Simultanious incubation caused a response dependent on the combination of the solvents. Incubation with n-hexane or 1-dodecanol exhibited an additive effect with ethanol, a decreased effect with butanol and hexanol, whereas butanol caused an increased inhibition when incubated with ethanol or hexanol. The results indicate that highly lipophilic solvents protect the enzyme by occupying hydrophobic areas in competition with amphiphilic substances. The supraadditive effect seen after incubation with butanol/n-hexanol or butanol/ethanol indicate a mechanism which comprises the hydrophilic interphase aswell as hydrophobic locations in the membrane.

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447P SECOND MESSENGER FUNCTION OF CALCIUM IN ATP-STIMULATED RAT PAROTID SALIVARY GLANDS

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It has recently been shown that ATP and related purines stimulate both an elevation of intracellular calcium ($[Ca^{2+}]_i$) and amylase release in rat parotid salivary glands (Gallacher, 1982; McMillian et al., 1987; Arkle et al., 1991). From their studies on lacrimal acinar cells Sasaki & Gallacher (1990) have suggested that the calcium-mobilising effect of ATP may differ from that seen with other calcium-dependent secretagogues for which a second messenger function of calcium is well established in that ATP may increase $[Ca^{2+}]_i$ via regulation of a receptor operated channel that is independent of inositol polyphosphates. In the work described here we have further investigated the putative second messenger function of calcium in ATP-stimulated parotid acini by determining the effects of calcium withdrawal and of calmodulin and protein kinase C inhibitors on ATP-dependent responses.

$[Ca^{2+}]_i$ was measured in isolated parotid acini by the Fura-2 fluorescence technique and amylase release was measured from parotid pieces by Bernfeld's method. ATP (3-1000μM) stimulated a dose-dependent increase in $[Ca^{2+}]_i$. The Fura-2 signal from 100μM-ATP-stimulated acini was unaffected by inclusion of 100μM-$MnCl_2$ (which quenches Fura-2 fluorescence) in incubation buffers (control, 430 ± 60; test, 420 ± 51nM $[Ca^{2+}]_i$, n=4 (mean ± s.e. mean)). When acini were preincubated for 5, 10 or 30 min. in media containing 2mM-EGTA and 1.4mM-$CaCl_2$, the 100μM-ATP-stimulated calcium signal was diminished (control, 448 ± 36; tests, 436 ± 42; 382 ± 28 and 343 ± 26nM $[Ca^{2+}]_i$ respectively, n=4) but not abolished. Preincubation of parotid pieces for 10 min. with either the protein kinase C inhibitor 1-(5-isoquinolinylsulphonyl)-2-methylpiperazine (H7) (100μM) or the calmodulin antagonists trifluoperazine (30μM) or N-(6-aminohexyl)-5-chloro-1-naphthalene sulphonamide (W7) (100μM) reduced the peak secretory response to 300μM-ATP from 23 ± 4 to 12 ± 2, 15 ± 2 and 11 ± 4 international units of amylase per min. per g. wet weight of tissue respectively (n=4).

These data lead us to conclude that ATP may activate secretion by mobilisation of calcium from both EGTA-sensitive and EGTA-insensitive (possibly intracellular) pools. This response is unlikely to be mediated by a non-selective increase in membrane permeability to cations as it was not accompanied by manganese entry into acini. The secretory effects of ATP may involve activation of calcium-calmodulin-dependent processes and of protein kinase C. These findings are consistent with a second messenger function of calcium in ATP-stimulated parotid acini.

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448P CHRONIC BARBITAL TREATMENT INCREASES DIHYDROPYRIDINE-SENSITIVE AND DIHYDROPYRIDINE-INSENSITIVE NEURONAL CALCIUM UPTAKE

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Acutely, barbiturates inhibit neuronal calcium uptake. We have shown that chronic barbiturate treatment increases the number of dihydropyridine binding sites in the CNS and decreases their affinity (Rabbani and Little, 1990a). These sites are thought to correspond to one type of voltage-sensitive calcium channel. The dihydropyridine calcium channel antagonist, nitrendipine, gave partial protection against the barbiturate withdrawal syndrome in vivo (Rabbani and Little, 1990b) We have now investigated functional changes in neuronal calcium channels after chronic barbiturate treatment, and the effects of nitrendipine and the calcium channel activator, Bay K 8644.

Male mice, TO strain, 20 - 25g, were used throughout. Barbital was given in powdered food for 7 days (mean intake: 400 mg/kg/24h on day 1, rising to 700 mg/kg/24h on day 7). Controls received the same amount of food without drug. Preparations of hippocampal slices and cortical synaptosomes were made 24h after withdrawal from barbital treatment, a time at which the behavioural withdrawal syndrome is maximal and tolerance occurs to the behavioural actions. Hippocampal slices were preincubated for 10 min, then exposed to $^{45}Ca^{2+}$ for 10 min with 5 or 70 mM K^+. Preincubation and incubation were made in the presence or absence of 10 μM nitrendipine, in Kreb's buffer gassed with 95% O_2 and 5% CO_2, at 37°C. After termination of uptake by cold lanthanum buffer, the tissues were homogenised and the $^{45}Ca^{2+}$ content counted. Synaptosomal membranes were prepared from cerebral cortex. After 10 min preincubation at 37°C in standard Krebs' buffer, in the presence or absence of nitrendipine (10 μM) and/or (±)-Bay K 8644 (500 nM), $^{45}Ca^{2+}$ uptake was initiated by addition of 5 or 70 mM K^+ buffer containing $^{45}Ca^{2+}$. Uptake was terminated 1 sec later with cold lanthanum buffer, and the tube contents filtered and the $^{45}Ca^{2+}$ content counted.

In both tissues the calcium uptake was significantly higher after barbital treatment than in control preparations . The increased uptake into the slices was blocked by nitrendipine, but the synaptosomal uptake was insensitive to either nitrendipine or Bay K 8644. Cadmium blocked uptake in both tissues.

Table 1: Uptake stimulated by increased K^+, nmol/mg protein, mean ± s.e.m. **P=0.01 slices, *P<0.02 synaptosomes, cf. controls and barbital, no drug, Student's 't' test, n = 7 for slices, 6 for synaptosomes, each from a different animal.

SLICES:	No drug	Nitrendipine
Controls	40.26±12.9	56.19±26.38
Barbital	151.0±34.2**	68.87±20.69

SYNAPTOSOMES	No drug	Nitrendipine	Bay K 8644	Nitr.+Bay K
Controls	5.75±0.36	5.6±0.3	6.06±0.29	5.75±0.3
Barbital	7.31±0.39*	7.26±0.42	7.52±0.45	7.67±0.48

The results suggest that chronic barbiturate treatment, that caused tolerance and a withdrawal syndrome in vivo, increases the activity of neuronal calcium channels sensitive to dihydropyridines and those that are insensitive.

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449P FELODIPINE, A CALCIUM ANTAGONIST WITH HIGH BRAIN PENETRATION AND NEUROPROTECTIVE ACTIVITY, AT DOSES LIKELY TO SHOW LITTLE CARDIOVASCULAR ACTIVITY

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Calcium antagonists are proposed as neuroprotective agents against ischaemia and may act neuronally reducing calcium influx associated with ischaemic cell death or on vasculature to increase cerebral blood flow. The calcium antagonist felodipine (Leonetti et al.,1984) was tested against global ischaemia in the gerbil. Ex-vivo binding studies were used to estimate brain penetration. Heart rate and blood pressure were measured in anaesthetised animals. Male gerbils (54-70 g) were used throughout.

Cardio-vascular studies. Animals (n=5-6)were anaesthetised with saffan followed by a bolus of chloralose (30mg/kg) and infusion of 15 mg/kg/h. Blood pressure and heart rate were recorded from the femoral artery, drug was given s.c. at 0.5 or 2 mg/kg. Both doses of drug induced hypotension and tachycardia. 0.5mg/kg reduced mean blood pressure by 10.5 % and increased mean heart rate by 29%. 2 mg/kg caused a 22% fall in blood pressure and a 96% increase in heart rate.
Ex-vivo binding. Brain penetration was assessed by injection of test compound (i.p.). After 30 min animals were sacrificed the brains rapidly removed and homogenised in cold buffer. Aliquots of homogenate were assayed with [^{3}H]-nitrendipine and specific binding determined by incubation with 1 uM nimodipine. Samples were incubated for 60 min and filtered over Whatman GF/B filters with ice cold saline washes. Brain penetration was assessed by comparison with a calibration curve of test compound given to saline treated animals. Comparison was made with nimodipine. Felodipine showed a greater potency (0.12 vs 0.94 mg/kg) and a greater penetration (2.5 ± 0.26% vs 0.81 ± 0.16 %) than nimodipine (n=11, 2 independent experiments).
Neuroprotection. Global ischaemia was induced by 5 min bilateral carotid occlusion under halothane anaesthesia. Drugs were administered (s.c.) 1 h after insult (n=8-10 per treatment) and histology performed 4 days later, area of hippocampal damage assessed and expressed as mm^2 ± s.e.mean. Felodipine was given in the dose range 0.01 - 0.5 mg/kg, MK-801 (1 mg/kg) was given to one group as a benchmark. Control damage (vehicle treated) was 5.35 ± 1.04. The damage in the MK-801 treated group was 2.14 ± 0.59 ($p<0.05$, Students' t-test). Felodipine showed a modest reduction in damage at the lowest dose tested (4.14 ± 1.21). This effect was substantiated by the next dose which produced significant protection (2.34 ± 0.59, $p<0.05$) similar to MK-801. Higher doses showed a return to control area of damage.

The cardio-vascular findings show that higher doses of felodipine are hypotensive and tachycardic, the compound penetrates the brain better than nimodipine and is neuroprotective at a dose with minimal cardio-vascular activity. This may indicate a neuronal action of the compound.

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450P TOLERANCE TO THE SEDATIVE, ATAXIC AND HYPOTHERMIC ACTIONS OF THE DIHYDROPYRIDINE CALCIUM CHANNEL ANTAGONIST, PN 200-110 (ISRADIPINE)

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The CNS contains high affinity binding sites for dihydropyridines, but these calcium channel antagonists have little effect on behaviour. They have some central depressant effects and potentiate the actions of ethanol. We have shown that PN 200-110 stereospecifically protects against the ethanol withdrawal syndrome when given acutely on cessation of ethanol intake (Littleton et al., 1990). When given chronically with ethanol, this dihydropyridine prevented the electrophysiological and behavioural manifestations of ethanol withdrawal. We have suggested that this latter effect is due to an adaptive response, decreasing the number of dihydropyridine binding sites, as shown by Panza et al. (1985) and opposing the increase in the number of these sites that is caused by chronic ethanol (Whittington et al., 1991). We have now studied the actions of racemic PN 200-110 when this compound is given by the chronic treatment schedule that we found to be effective in our ethanol studies.

Male mice, TO strain, 30 - 35g, received PN 200-110, 10 mg/kg i.p., twice daily for 13 days. Controls received concurrent vehicle injections. The effects of PN 200-110 were measured 12h after the last chronic injections. Spontaneous locomotor activity was measured by infra-red beam interruption (n=12 pairs per group), ataxia by the ability to stay on a rotating rod (n=12 mice per group) and body temperature by rectal probe (n=9 mice per group). Withdrawal was measured by ratings of convulsive behaviour on handling (Littleton et al., 1990).

Tolerance was seen to the sedative and hypothermic effects of PN 200-110, and to a less extent to the ataxic actions:

Measurement/time	Chr. veh/acute veh	chr. veh/acute PN	chr. PN/acute PN	chr. PN/acute veh.
Locomotor counts, 30 min total	9248 ± 492	2279 ± 173 *	4466 ± 324 †	8431 ± 557
Rotorod time (sec), at 15 min	180 ± 0 (cut-off point)	118 ± 23 *	150 ± 16	180 ± 0 (cut-off point)
Temperature (°C), at 20 min	37.8 ± 0.2	35.5 ± 0.4 *	37.2 ± 0.1 †	37.8 ± 0.2

(PN = PN 200-110, 10 mg/kg i.p.; veh. = vehicle; chr. = chronic; times are from acute injections, at maximum effects)
* $P<0.01$ cf. chr. veh/acute veh; † $P<0.01$ cf. chr. veh/acute PN, comparisons by Student's t-test for the locomotor counts and body temperature and nonparametric analysis of variance for the rotorod results.
No signs of withdrawal hyperexcitability were seen between 6h to 36h after the last chronic injections of PN 200-110.

The results show that tolerance occurs to these actions of PN 200-110. Increased metabolism cannot yet be excluded, although tolerance does not appear to occur to the cardiovascular effects (Pedersen et al., 1989). The ataxic and sedative actions are thought to be due to neuronal actions, but the hypothermia may also involve vascular channels.

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451P CHOLECYSTOKININ-OCTAPEPTIDE STIMULATES ACTH RELEASE FROM AtT-20 TUMOUR CELLS THROUGH A MECHANISM INDEPENDENT OF POLYPHOSPHOINOSITIDE HYDROLYSIS

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Cholecystokinin-octapeptide (CCK-8) has been found to be present in the hypothalamus (Beinfeld *et al,* 1981) where it was suggested to co-exist with corticotrophic-releasing factor (CRF) in medial parvocellular neurons in the paraventricular nucleus (Mezey *et al,* 1985). We investigated whether CCK-8 could induce secretion of adrenocorticotrophic hormone (ACTH) from the clonal mouse AtT-20 anterior pituitary tumour cell line, and to see if any release may involve CCK-8-stimulated inositol phosphate production. Culture of AtT-20/D16-16 cells and radioimmunoassay for ACTH were performed as previously described (Hook *et al,* 1982). For measurement of inositol phosphates, AtT-20 cells were preincubated for 48 h in inositol-free culture medium supplemented with 2 μCi/ml [^{3}H]-inositol. Washed, harvested, labelled cells were incubated at a cell density of 10^6 cells/ml in DMEM + 0.25% BSA + 25 mM HEPES + 3 μg/ml bacitracin + 10 mM LiCl. After drug challenge for 0-60 min at 37°C, reactions were stopped by addition of (1:2) chloroform/methanol and vortexed. After 20 min, organic and aqueous phases were separated by addition of (1:1) chloroform/H_2O then centrifugation (1000 g, 5 min). The organic phase was sampled for radioactivity and the inositol phosphate contained in the aqueous phase (4°C) were separated on Dowex-formate anion-exchange columns. The radioactivity incorporated into inositol, IP_1, IP_2, IP_3 and IP_4 was separated by successive elutions with H_2O then with 0.1 M formic acid + 0.2, 0.4, 0.8 and 1.2 M ammonium formate respectively. As expected, CRF (0.1 μM) and forskolin (20 μM) stimulated ACTH release from AtT-20 cells. Likewise, CCK-8 could also stimulate ACTH secretion in a dose-dependent fashion (EC_{50} = 20 nM) and 1 μM CCK-8 induced a release rate of 133 ± 34 pg ACTH/10^6 cells/h, mean ± s.e.m., n = 12 (4.4 fold increase over basal release). Exposure of AtT-20 cells to high (6.8 mM) Ca^{2+} concentrations in the presence of Li^+ caused a time-dependent reduction in radioactivity of the lipid- and inositol-content of the cells at 60 min to 50 ± 10 and 44 ± 6% of unstimulated levels (mean ± s.e.m., n = 3) with a concomitant increase in IP_1 levels to 250 ± 10% of control (n = 3) at 60 min, and a peak rise in IP_2 levels to 350 ± 25% of unstimulated levels (n = 3) at 10 min. Over the concentration range 10^{-11} to 10^{-6} M, CCK-8 (preincubated for up to 60 min) and in the presence of Li^+ produced no marked change in inositol phosphates, inositol or lipid-radioactivities (n = 3)

These results further indicate a role for CCK-8 in ACTH release from corticotrophs. However, although phosphoinositide hydrolysis in the cells could be detected by exposure to high Ca^{2+}, no detectable change in phosphoinositide hydrolysis could be seen with CCK-8. Therefore, in AtT-20 cells, CCK-8 acts as a secretagogue but produces its effects by a cyclic AMP-independent (Reisine *et al,* 1985) and an inositol phosphate-independent mechanism.

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452P CHARACTERISATION OF [^{3}H]DIMETHYLSTAUROSPORINE BINDING SITES BY DISPLACEMENT STUDIES USING PROTEIN KINASE C INHIBITORS

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Staurosporine inhibits protein kinase C (PKC) activity by interacting with, or close to, its ATP binding site (Nakadate, *et al,* 1988). Since the ATP binding sites of different serine/threonine- and tyrosine-specific kinases show homology, staurosporine has only modest selectivity for PKC (Rüegg & Burgess, 1989) suggesting that its radiolabelled derivative, [^{3}H]N,N-dimethylstaurosporine ([^{3}H]DMS), is a useful tool for characterisation of different classes of protein kinase within a tissue. We have characterised [^{3}H]DMS binding sites in rat midbrain and lung cytosol by displacement studies using the PKC inhibitors, H7 and staurosporine.

Adult male rat midbrain or lung was homogenised in 20mM Tris-HCl (pH7.5) with 50mM EtSH ,2mM EDTA, and 1mM phenylmethylsulphonyl fluoride, then centrifuged (12,000g, 4°C, 20 mins). The supernatant was recentrifuged (12,000g, 4°C, 5mins) and this supernatant taken to represent cytosol. The binding assay constituents were 50mM Tris-HCl (pH8.0), 1μg/ml bovine gamma globulin (BGG), 1mM dithiothreitol, [^{3}H]DMS (5nM), H7 or staurosporine and cytosol (Gross, *et al,* 1990). Total binding was measured in the absence of PKC inhibitor and non-specific binding (which was approximately 25% of total binding) was defined in the presence of 3μM staurosporine. After a 30 min incubation (4°C), the protein was precipitated at 0°C with 1mg/ml BGG and 10% polyethyleneglycol 8000 and pelleted by centrifugation (12,000g, 4°C, 5 mins). The pellet was washed once with 1ml of ice cold Tris-HCl then counted. Staurosporine displaced specific [^{3}H]DMS binding to sites in lung and midbrain cytosol with Hill slopes of 0.58±0.07 and 0.22±0.05 respectively, consistent with both tissues having more than one site with different affinities for staurosporine. Binding to midbrain sites was more sensitive to displacement by staurosporine (IC_{50}=0.23±0.06nM) than lung sites (IC_{50}=9.38±1.93nM). Midbrain cytosol contained sites sensitive to very low concentrations of staurosporine (with 27±4% displacement at just 0.02nM), that were apparently absent in lung. The H7 displacement curves were similar in both tissues. However, the potency of H7 was quite low with concentrations up to 500μM displacing only approximately 40% of total binding.These results are consistent with both lung and midbrain cytosol containing more than one kinase type which differ in their affinities for staurosporine. Midbrain, but not lung, appears to express a kinase (or set of kinases) which is highly sensitive to staurosporine,much more so than has previously been reported for staurosporine on purified kinase activity (Gross, *et al,* 1990). However, H7 was unable to distinguish any sites unique to either tissue. The weak displacement of binding by H7 suggests that the PKC recognition sites for staurosporine and H7 are not identical.

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453P THE EFFECTS OF DIFFERENT CLASSES OF ACTIVATORS OF PROTEIN KINASE C ON RAT MIDBRAIN

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It is now known that there are at least 7 distinct isoforms of protein kinase C (PKC) (Nishizuka, 1988) and messenger RNAs for all these isoforms have been detected in rat midbrain (Scott Young III, 1989). We have been interested for some time in the possibility that these isoforms may exhibit selective pharmacological properties. This idea is supported by the differential effects of PKC inhibitors on phorbol ester-induced hormone release from pituitary (Johnson & Mitchell, 1989) and the selectivity of some PKC activators on regulation of Ca^{2+} channels in these cells (MacEwan *et al,* 1990).

To directly examine the properties of the kinases, we used a mixed-micelle histone III-S kinase assay similar to that of Huang *et al* (1988). Phosphatidyl serine-dependent kinase activity from male rat brain cytosol (partially-purified on DEAE cellulose) was measured in the presence of 100 μM or zero (< 3 nM) free Ca^{2+}. Phorbol 12,13-dibutyrate (PDBu) activated Ca^{2+}-dependent PKCs with a consensus EC_{50} of 21 ± 5 nM compared to that for Ca-independent activity of 790 ± 120 nM (means ± s.e. mean, n = 8 - 20). Phorbol 12-myristate 13-acetate induced activity with similar maxima in each case and with a potency on Ca^{2+}-dependent activity around 10 fold greater than on Ca^{2+}-independent. Two compounds which showed selectivity in the modulation of Ca^{2+} channels by apparently distinct PKCs (MacEwan *et al,* 1991) were also tested. Deoxyphorbol 13-isobutyrate recruited similar maximal activity of Ca^{2+}-independent kinases (93 ± 3% of that due to PDBu), but rather less (73 ± 5%) of the maximal PDBu-evoked Ca^{2+}-dependent activity (mean ± s.e. mean, n = 8 - 9). Mezerein recruited 82 ± 5% of the maximal Ca^{2+}-independent activity evoked by PDBu but only 37 ± 5% of the Ca^{2+}-dependent increment (mean ± s.e. mean, n = 5 - 9). The diglyceride 1,2-dioctanoyl-sn-glycerol (DOG) activated the overall population of kinases with a consensus EC_{50} of 0.8 ± 0.3 μM in the presence of 100 μM Ca^{2+}. However, even at high concentrations (up to 1 mM), DOG could only elicit about half of the maximal Ca^{2+}-independent activity seen with PDBu (n = 4 - 8).

These data indicate that activity of Ca^{2+}-dependent and independent PKCs can be differentially elicited by some PKC activators. Under certain conditions, the diglyceride, (DOG) appears to be ineffective on a component of the Ca^{2+}-independent activity whereas other compounds such as mezerein may be ineffective on some of the Ca^{2+}-dependent kinases.

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454P REGIONAL DIFFERENCES IN THE AFFINITY OF DIACYLGLYCEROL ANALOGUES FOR [^{3}H]-PHORBOL 12,13-DIBUTYRATE BINDING SITES

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Diacylglycerols are one of the endogenous activators of protein kinase C (PKC) (Nishizuka, 1988). We have previously postulated that the short chain diacylglycerol, 1,2-dioctanoyl *sn*-glycerol (DOG) may selectively activate an isoform(s) of PKC (Johnson *et al,* 1989). Since phorbol esters, such as phorbol 12,13-dibutyrate (PDBu), are known to activate PKC by acting at the diacylglycerol binding site, we investigated the effect of a range of 1,2-diglycerides on cytosolic [^{3}H]-PDBu binding from various regions in the rat with known contents of the different Ca^{2+}-dependent PKC-isoforms.

Cytosolic [^{3}H]-PDBu binding was performed as previously described (MacEwan & Mitchell, 1990). The range of 1,2-diglycerides consisted of unmixed saturated chains of 6 -18 carbon atoms ($C_{6:0}$ - $C_{18:0}$), 1,2-dioleoyl *sn*-glycerol (DO) ($C_{18:1}$, cis-9) which has two unmixed chains with one unsaturated double bond in each, and a mixed unsaturated chain diacylglycerol, 1-stearoyl-2-arachidonoyl *sn*-glycerol (SAG) ($C_{18:0}/C_{20:4}$,[cis,cis,cis,cis]-5,8,11,14). When measuring the IC_{50} for reversal of specific binding by 0 - 500 μM of each compound in rat lung, frontal cerebral cortex and cerebellum, DO and SAG showed the highest affinities, which were similar in each of the three regions tested (11 - 23 μM with DO, and 6, 4 and 4 μM with SAG for lung, cortex and cerebellum respectively (n = 4)). However, the IC_{50} values for $C_{6:0}$ - $C_{18:0}$ saturated diglycerides varied considerably, with their affinities in lung being consistently lower than in the other regions for all the chain lengths tested. Differences in affinity were not due to selective actions of lipases because DOG pre-incubated in lung had the same subsequent affinity for binding in cortex as DOG pre-incubated in cortex or cerebellum. Plots of affinity against chain length were bell-shaped and showed lowest affinity for [^{3}H]-PDBu-binding with $C_{6:0}$ and $C_{18:0}$ (IC_{50}s were 50 - 176 μM for $C_{6:0}$, $C_{8:0}$ and $C_{18:0}$ in cortex and cerebellum and 1120, 1354 and 416 μM in lung respectively). The highest affinity in the saturated diglyceride series was with a C_{14} chain length (IC_{50}s of 17 μM for cortex and cerebellum; 46 μM in lung). Using cytosol from various tissues with known contents of Ca^{2+}-dependent PKC-isoforms (Shearman *et al,* 1988), the affinity of DOG varied not only using lung, cortex and cerebellum, but with all the regions tested and an inverse relationship was apparent between the affinity for [^{3}H]-PDBu binding and the proportion of α-PKC isoform present in the tissue.

There is clearly an optimal chain length and composition for diacylglycerol interaction with [^{3}H]-PDBu binding sites, but it now appears that short chain saturated diglycerides such as DOG may selectively interact with certain isoforms of PKC.

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455P THE INHIBITORY EFFECTS OF HOE 140 IN MOUSE ABDOMINAL CONSTRICTION ASSAYS

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Antagonists selective for the bradykinin BK_2 receptor have been shown to possess antinociceptive properties (Steranka et al., 1988). In contrast to earlier bradykinin antagonists which are rapidly destroyed in vivo, the novel peptide HOE 140 (D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin) has a long duration of action in vivo (Wirth et al., 1991) making it a valuable tool for investigating the role of the BK_2 receptor. In this study the ability of subcutaneously administered HOE 140 to inhibit the abdominal constriction response induced by intraperitoneal acetic acid (0.25%), kaolin (3mg), zymosan (1mg), bradykinin (BK) (10μg), and by BK (1μg) in mice pre-treated with PGE_2 (100ng i.p.) was investigated. All agents were administered to groups of at least 10 mice in a volume of 0.2 ml/10 g body weight. The doses of HOE 140 used ranged from 0.3 to 1000 μg/kg with a 30 min pre-treatment time. With the exception of those groups challenged with BK, the responses were counted using a micro-computer which also recorded the time at which each response occurred. The BK treated groups were observed for 30 s following challenge, with the results expressed as the percentage of animals responding within this period.

HOE 140 abolished the response to BK (ED_{50} 1.92 μg/kg; 95% conf. limits 0.93-3.97), and also that of BK in animals pre-treated with PGE_2 (ED_{50} 3.65 μg/kg; 2.69-4.85). The maximal inhibitory effect following challenge with acetic acid and kaolin was 70% (ED_{25} 16.03 μg/kg; 7.8-24.4) and 59% (ED_{25} 2.7 μg/kg; 1.97-3.60) respectively. No significant inhibition of the response to zymosan was seen at doses up to 1 mg/kg. Analysis of the time distribution of the abdominal constrictions over the 15 min observation period revealed that 0.25% acetic acid evoked a bi-phasic response with an initial peak at 4-5 min followed by a second prolonged phase starting at 8 min post injection. In contrast, kaolin and zymosan evoked single phase responses with peaks at 5 and 6 min respectively. HOE 140 depressed both phases of the acetic acid response, whereas a potent inhibition of the kaolin response was only seen during the initial 6 min. The results indicate that HOE 140 is a potent antagonist in vivo of nociceptive responses evoked by BK. However, the HOE 140 sensitive component varies considerably between responses evoked by the other algesic agents used. In addition, a clear temporal distinction within the response to kaolin shows that in this case only the initial part is dependent upon BK formation.

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456P L-689,660, A FUNCTIONALLY SELECTIVE AGONIST AT M_1 AND M_3 MUSCARINIC RECEPTORS

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The synthesis of a novel series of low efficacy cholinomimetics has recently been described (Baker *et al* 1991). The pharmacological profile of one of these compounds, L-689,660, (1-azabicyclo (2.2.2) octane, 3-(6-chloropyrazinyl) maleate) has been determined in comparison with the muscarinic agonists AF102B (cis-2-methylspiro-(1,3,oxathiolane 5,3')-quinuclidine hydrochloride) and RS-86 (2-ethyl 8-methyl-2,8-diazaspiro [4,5] decan 1,3-dione hydrochloride).

Assay		L-689,660 (R-enantiomer)	AF102B (Racemic)	RS-86 (Achiral)
Rat Cortex Binding	[^{3}H]-N-Methyl-Scopolamine (NMS) Kapp (μM)[a]	0.037	3.1	5.0
	[^{3}H]-Oxotremorine-M (Oxo-M) Kapp (μM)[a]	0.0013	0.078	0.04
	NMS/OXO-M Ratio	28	39	130
M_1 Rat SCG (Pirenzepine sensitive depolarization) EC_{50} μM; (RM)[b]		0.05 (1.2)	8.8 (1.3)	1.0 (1.0)
M_2 Guinea-pig paced atria (-ve inotropy) [pA_2] EC_{50} μM; (RM)[c]		[7.2]	[5.2]	0.5 (1.0)
M_3 Guinea-pig ileum (Contraction) EC_{50} μM; (RM)[c]		0.03 (0.6)	5.6 (0.3)	2.7 (1.0)
M_3 Guinea-pig trachea (Contraction) EC_{50} μM; (RM)[c]		0.03 (0.6)	4.6 (0.4)	0.4 (1.0)

[a] Apparent affinity constant (Kapp) corrected for ligand occupancy
RM: Relative to the maximum response to 1μM (±) muscarine (RM)[b] or carbachol (RM)[c].

L-689,660 also caused a dose-related increase in electrically-stimulated acetylcholine (ACh) release from rat hippocampal slices consistent with antagonism at terminal muscarinic autoreceptors. The results show that L-689,660 had 100 fold higher affinity for cortical muscarinic receptors than AF102B and RS-86. The NMS/Oxo-M binding ratios for L-689,660 and AF102B are indicative of weak partial agonists whilst the ratio for RS-86 is similar to that for pilocarpine (Freedman *et al* 1988). At M_1 and M_3 receptors L-689,660 was a potent agonist although on the ileum and trachea it was not fully efficacious. In contrast at M_2 receptors on the atria L-689,660 lacked efficacy. AF102B was approximately 100 times less active than L-689,660 in isolated tissues. On the ileum AF102B was also found to antagonise carbachol (pA_2 [5.5]) but the slope of the Arunlakshana and Schild plot was 0.5. On the atria, AF102B also showed some agonist activity (EC_{50} 10μM RM 0.3). This shows the partial agonist nature of AF102B or that its separate enantiomers may have different intrinsic activities. RS-86 was fully efficacious in M_1, M_2 and M_3 assays. These studies show that low efficacy cholinomimetics such as L-689,660 display functional muscarinic receptor subtype selectivity.

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457P THE PHARMACOLOGY *IN VIVO* OF L-689,660, A FUNCTIONALLY SELECTIVE M_1/M_3 MUSCARINIC AGONIST

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The low efficacy cholinomimetic L-689,660 (1-azabicyclo (2.2.2) octane, 3-(6-chloropyrazinyl) maleate) has been shown to be a functionally selective M_1/M_3 muscarinic receptor agonist which displays antagonism at the M_2 muscarinic receptor sub-type (Hargreaves *et al* this meeting). L-689,660 has been evaluated in several assays *in vivo* and in some compared directly with the muscarinic agonists AF102B and RS-86.

Administration of L-689,660 produced signs of peripheral (salivation) and central (hypothermia) cholinergic stimulation (ED_{100} 0.1mg/kg i.p.; ED_{50} 0.3mg/kg i.p. respectively) that were less marked than after RS-86 (ED_{100} 0.73mg/kg i.p.; ED_{50} 1.7mg/kg i.p.) reflecting its lower muscarinic efficacy. Peripheral and central cholinergic signs were not seen until after relatively high doses of AF102B (ED_{100} 30mg/kg i.p.; ED_{50} >30mg/kg i.p. respectively) indicating a lower potency than L-689,660. The brain penetration of L-689,660, AF102B and RS-86 was determined using the technique of *ex vivo* binding (Freedman *et al* 1989). L-689,660 penetrated the brain well and had an ED_{50} (dose producing 50% inhibition of specific binding) of 0.86mg/kg i.p. At 30 min after administration 0.93±0.2% of the L-689,660 dose was in brain. In contrast AF-102B was 30 fold weaker than L-689,660 (ED_{50} 28mg/kg i.p.) with brain penetration of 0.57±0.02%. Data for RS-86 in the *ex vivo* binding assay were ED_{50} 11mg/kg i.p. with % penetration of 1.5±0.3%.

In urethane anaesthetised rats L-689,660 (0.1mg/kg i.v.) produced a CNS rhythmic slow wave (theta rhythm) characteristic of muscarinic agonists that was antagonised by scopolamine. AF102B also induced a transient theta rhythm but at the higher dose of 10mg/kg in line with its lower brain penetration and affinity at central muscarinic receptors.

Cardiovascular studies in conscious rats showed that L-689,660 (1-1000µg/kg i.v.) and AF102B (1-3000µg/kg i.v.) have similar effects on mean arterial blood pressure (MABP). The predominant effect was a transient fall (-25%) in MABP. The hypotensive effects of AF102B were shifted 100 fold rightward of L-689,660 in line with its lower potency at M_3 receptors *in vitro*. High doses of AF102B (>100µg/kg) produced a dose-related fall in heart rate that was blocked by N-methylscopolamine (NMS; 0.5mg/kg i.v.), confirming the partial agonist nature of racemic AF102B that was detected on the isolated atria *in vitro*. In contrast, a modest tachycardia was observed in conscious rats with L-689,660 due probably to its M_2 antagonist properties. Tachycardia was more pronounced in urethane-anaesthetised rats where vagal tone is high. The cardiovascular profile for RS-86 was typical of a non-selective muscarinic agonist. RS-86 (1-1000µg/kg i.v.) caused a transient peripherally mediated fall in MABP and heart rate followed by a reflex tachycardia and centrally driven increase in MABP and heart rate. Peripheral effects were blocked selectively by NMS (0.5mg/kg i.v.) and the central action by atropine (1.0mg/kg i.v.). In conclusion L-689,660, a selective M_1/M_3 muscarinic agonist, is more potent than AF102B and has an improved cardiovascular side-effect profile over non-selective muscarinic agonists such as RS-86.

Freedman, S.B., Harley, E.A. and Patel, S. (1989) Eur. J. Pharmacol. 174, 253-260

458P THE EFFECTS OF A NOVEL MUSCARINIC RECEPTOR AGONIST, L-689,660 IN THE MOUSE-TAIL FLICK TEST OF ANTINOCICEPTION

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It has been suggested that cholinergic antinociception is mediated via muscarinic receptors in the CNS independently of the opiate system, although the subtype(s) involved have not been positively identified (Ghelardini *et al* 1990). Baker *et al.* (1991) have described a novel series of low efficacy muscarinic receptor agonists one of which, L-689,660 (1-azabicyclo (2.2.2) octane, (3-(6-chloropyrazinly) malate, R-enantiomer) has an agonist action at M_1 and M_3 receptors and an antagonist action at M_2 receptors (Hargreaves *et al.* this meeting). In the present study we have examined the antinociceptive properties of L-689,660.

The mean baseline tail-flick latency (TFL) to radiant heat was determined in male BKTO mice (22-40g, Bantin & Kingman, England) before drug administration and at 2, 10 and 20 minutes after i.p. injection of L-689,660. Morphine (16.0 mg/kg, i.p.) was included as a positive control. Exposure to the heat source was limited to 8 s and results were expressed as a percentage of the maximum possible effect (MPE = (TFL - Baseline TFL)/(8 -Baseline TFL) x 100). Results were analysed by analysis of variance and *post hoc* Tukey tests.

L-689,660 induced a dose and time dependent antinociceptive effect with a minimum effective dose (MED) of 0.03 mg/kg (20 mins post injection: MPE = 3.3 ± 16.2%). However, doses of 0.3 and 1.0 mg/kg of L-689,660 and 16 mg/kg of morphine induced antinociception 10 mins after i.p. administration ($p<0.05$ *vs* vehicle control) with mean MPE's of 81.5 ± 9.9%; 59.7± 16.0% and 67.0 ± 13.0% respectively. The antinociceptive effect of L-689,660 (0.3mg/kg) was blocked by pretreatment with scopolamine (0.4 mg/kg, i.p. 20 minutes beforehand), but not by N-methyl scopolamine (0.4 mg/kg, i.p.) or naloxone (3.0 mg/kg, i.p.) indicating that the antinociceptive effect was mediated by muscarinic receptors located in the CNS and independently of the opiate system. L-689,660, at a dose of 0.3 mg/kg (i.p.) induced significant antinociception up to 200 min after administration (MPE = 37.1 ± 12.5%), whereas the antinociception induced by 16.0 mg/kg (i.p.) of morphine returned to baseline control levels 180 min after administration ($p<0.05$ *vs* vehicle control). The antinociceptive effects of the non-selective muscarinic receptor agonist, RS86 was also examined. RS86 increased significantly tail-flick latencies, although the compound was about 10 fold weaker than L-689,660 (MED = 0.3 mg/kg, i.p., MPE = 71.3, ± 15.5%), which are in agreement with the rank order potencies of the agonist actions of L-689,660 and RS86 at both M_1 and M_3 receptors (Hargreaves *et al.*, this meeting)

In conclusion, L-689,660 induces a long lasting antinociception in the mouse tail-flick test which may be mediated via M_1 or M_3, but not M_2 muscarinic receptors.

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459P ANTI-MUSCARINIC EFFECTS OF SOME GANGLIONIC NICOTINIC RECEPTOR AGONISTS IN GUINEA-PIG OLFACTORY CORTEX SLICES

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Electrophysiological studies have demonstrated the existence of both muscarinic and nicotinic receptors on mammalian cortical neurones (Krnjević & Phillis, 1963). Activation of cortical muscarinic receptors generally results in a slow and prolonged neuronal excitation, whereas nicotinic receptor stimulation evokes only weak excitatory effects (Krnjević, 1975). We have previously shown that the inhibitory effects of muscarinic agonists on excitatory neurotransmission in the olfactory cortex slice are mediated presynaptically via M_1-type muscarinic receptors located on the lateral olfactory tract and/or association fibre nerve terminals (Bagetta & Constanti, 1991). We have now re-investigated the possible existence of functional pre-or postsynaptic nicotinic receptor sites in guinea-pig olfactory cortex slices maintained in vitro, using extracellular and intracellular recording techniques. Carbachol (CCh, 25-150 µM, 2 min) produced a reversible inhibition of the surface N-wave as previously described. By contrast, 2 min bath-applications of nicotinic agonists e.g. (-)nicotine, DMPP, L-lobeline, (-)cytisine, tetramethylammonium bromide (TMA) or suberyldicholine (all at 50-100 µM, n=8 slices) consistently failed to affect the amplitude of the evoked field potential. Pre-incubation of slices for 15-30 min with 10-50 µM DMPP or lobeline however, produced a significant inhibition of subsequent CCh responses. By comparison, nicotine, cytisine, suberyldicholine or TMA induced a smaller and less consistent reduction of CCh effects. The anti-muscarinic effects of DMPP or lobeline were unaffected by d-tubocurarine (20 µM, n=4) or hexamethonium (20 µM, n=4). CCh dose-response lines were displaced in a parallel manner by DMPP or lobeline, suggesting a competitive-type antagonism. Schild plot analysis (Schild slope constrained to unity) of pooled data yielded pA_2 values of 5.58 for DMPP (n=6 slices) and 5.63 for lobeline (n=5 slices) respectively. Combination of 10 µM DMPP with 5 nM atropine produced a dose ratio (DR) shift (9.2 ± 2, n=3) close to the value (7.3) predicted for the interaction of two competitive antagonists. DMPP or atropine alone produced DR shifts of 4.1 ± 1, n=3 and 4.2 ± 0.4, n=3 respectively (means ± S.E.M.). Slow depolarizing responses to 75 µM CCh recorded intracellularly in olfactory cortical neurones (n=8 cells) were also reversibly abolished in the presence of 50 µM DMPP or lobeline, which themselves had little effect on neuronal membrane properties.

In conclusion, our experiments suggest that some ganglionic nicotinic receptor agonists exhibit significant cortical anti-muscarinic effects, which might be due to a direct atropine-like mechanism (c.f. Barlow & Franks, 1971).

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460P ATYPICAL $GABA_A$ RECEPTORS APPEAR TO MEDIATE INHIBITION OF EVOKED TWITCHES IN THE MOUSE VAS DEFERENS

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In a recent communication to this society (Taberner & Watson,1990) evidence for the presence of GABA B receptors in the mouse isolated vas deferens was presented, based on the ability of the GABA B agonist baclofen to inhibit electrically evoked twitches and the lack of activity of the GABA A drugs, muscimol and bicuculline. However, the maximum baclofen inhibition (18%) was half that of GABA and was very variable. Since there is some doubt as to whether baclofen is effective in this tissue (Evans, 1979, Bowery et al., 1981), we have repeated these experiments on a larger number of animals from 2 different strains of mice.

Vasa from adult LACG and MF1 mice were set up singly under 250mg tension in Mg^{++}-free Krebs Ringer bicarbonate medium (pH 7.4) gassed with 95% O_2, 5% CO_2. Isometric responses to field stimulation by single pulses (60-100V, 0.1Hz, 0.5 msec) were recorded using standard procedures. In low (9 mM) chloride medium NaCl was replaced with equimolar sodium isethionate. IC50 values and maximum inhibitory responses were derived by fitting cumulative dose response curves to the logistic expression.

All 18 preparations (12 LACG, 6 MF1) responded to bath applied GABA and 3-aminopropane sulphonic acid (3-APS). Mean IC50 values were 3.42 ± 0.27 (sem) and 4.23 ± 0.39 µM respectively for GABA, and 6.28 ± 1.7 and 6.78 ± 1.9 for 3-APS. Maximum inhibition ranged from 24 to 55% of the control response. GABA and 3-APS inhibition was completely abolished in low chloride medium. Only one LACG preparation responded to baclofen over the dose range 0.1 - 100 µM. The IC50 value was 3.0 + 0.3 µM (mean ± SEM of 6 replicates). GABA inhibition could not be blocked by adding bicuculline or picrotoxin (10 - 250 µM).

These results confirm the lack of baclofen response reported by Evans (1979) and suggest that the GABA receptors which regulate the evoked release of noradrenaline from sympathetic neurones in the mouse vas deferens are chloride-dependent GABA A. Since bicuculline and picrotoxin did not block this GABA-mediated inhibition it is possible that the receptors are not typical GABA A, although Bartholini et al (1990) have suggested that bicuculline does have non-GABA A-mediated excitatory effects in this preparation. We would conclude that our earlier finding of an inhibitory response to baclofen is not reproducible in the mouse vas deferens.

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461P EFFECTS ON GABA-MEDIATED NEUROTRANSMISSION OF RU33368, A BENZODIAZEPINE RECEPTOR AGONIST WITH MICROMOLAR AFFINITY BUT POTENT ACTIVITY *IN VIVO*

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RU33368, 6-ethyl-7-methoxy-5-methyl-2-(5'-methyl [1,3,4] oxadiazol-2'-yl) imidazo [1,2-] pyrimidine, is a new member of a series of selective benzodiazepine receptor ligands (Gardner, 1989). It showed low affinity in displacing ^{3}H-flunitrazepam binding from rat forebrain membrane (IC_{50}=6.3μM) but was potent in releasing 'punished-responding' in a rat conflict test (minimal effective dose = 5mg/kg p.o.); an effect that was blocked by flumazenil (20 mg/kg i.p.).

RU33368 inhibited leptazol-induced seizures in mice (ED_{50} with 95% confidence limits = 20 (13-32) mg/kg p.o.) but had weak or no effects in models of sedation or muscle relaxation. In the rotating drum test in rats the ED_{50} value was 90 (20-416) mg/kg p.o. and RU33368 was not effective at doses up to 200 mg/kg p.o. in the rotatating drum test in mice or the pull up test in rats.

The efficacy of RU33368 at bendodiazepine receptors was studied *in vitro* via the modulation of GABA-mediated synaptic inhibition of Purkinje cells in isolated cerebellar slices of rat (Hussain *et al.* 1991). Stimulation of the superficial layer (single pulses, 0.04 - 0.1 mA, 0.1 msec) evoked inhibition of spontaneous firing of Purkinje cells recorded with extracellular microelectrodes in the same folium. Inhibitions evoked by just-suprathreshold stimuli and analysed using peri-stimulus-time histograms were markedly potentiated by the benzodiazepine full agonist RU32007 at 1 μM (40-200%) and 10 μM (100-500%). RU33368 potentiated inhibition at 10 μM (40-400%) but not at 1 μM. The potentiation by both compounds was blocked by flumazenil (10μM).

The requirement of higher concentrations of RU33368 for effects in cerebellar slices parallels its low affinity for benzodiazepine receptor binding *in vitro*. If the kinetics and metabolism of RU33368 are similar to its 5'-methyl-1,2,4-oxadiazol-3'yl analogue, RU33203, it is unlikely that it acts via a higher affinity metabolite, but rather by achieving μg/ml levels of the parent in the brain (Gardner, 1990). It remains to be determined whether the lack of sedative and muscle relaxant properties of RU33368 results from a still insufficient effective concentration in the brain or from partial agonist properties of the compound.

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462P EFFECTS OF INTRACEREBROVENTRICULAR TAURINE, HOMOTAURINE AND GABA ON STRIATAL DOPAMINE METABOLISM

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Previously we found that intracerebroventricular taurine, GABA and homotaurine, a structural analogue of both taurine and GABA, elevate the concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in rat striatum and limbic forebrain. It was suggested that these amino acids reduce the release of DA and increase its synthesis (Ahtee & Vahala, 1985). To study this problem further we estimated the effects of these amino acids on striatal DA and its metabolites, including 3-methoxytyramine (3-MT), which is thought to reflect the rate of impulse-mediated DA release to synaptic cleft.

Amino acids (10 or 36 μmol/rat) were injected into the lateral brain ventricles of male Wistar rats. The rats given the 10 μmol dose were decapitated 15 min after starting the injection. The dose of 36 μmol was given in two equal portions at 30 min intervals, and the animals were decapitated 60 min after starting the first injection. The control rats were given similar volumes of saline instead of test solutions. Striatal concentrations of DA, DOPAC, HVA and 3-MT were assayed by high-performance liquid chromatography using electrochemical detection.

At the dose of 36 μmol/rat homotaurine, but not the other two amino acids, elevated striatal DA. All amino acids elevated dose-dependently striatal DOPAC; homotaurine was more effective than taurine or GABA. The most strikingt striking effect was the 40% decrease of striatal 3-MT already at the dose of 10 μmol/rat. At the 36 μmol/rat taurine and homotaurine reduced the 3-MT content by about 70%, whereas increasing the dose of GABA did not further reduce 3-MT. Striatal HVA was not clearly altered.

The fall of 3-MT concentration gives further support for previous suggestion that taurine and homotaurine similarly to GABA inhibit the impulse-related release of DA from striatal dopaminergic neurons (Garcia de Yebenes Prous et al., 1978; Ahtee & Vahala, 1985). DOPAC is thought at least partially to be formed intraneuronally, and reflect the rate of DA synthesis. Thus the finding that DOPAC is elevated suggests that the amino acids studied enhance the synthesis of DA. In conclusion, taurine and homotaurine similarly to GABA seem to reduce the release and consequently increase the synthesis of DA in rat striatum in the rank order of potency: homotaurine > taurine > GABA.

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463P HANDLING OF RATS FOR 28 DAYS CHANGES PERFORMANCE IN THE ELEVATED PLUS-MAZE WITHOUT ALTERING [^{3}H]-FLUNITRAZEPAM BINDING TO FRONTAL CORTICAL MEMBRANES

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It has been recently reported that repeated handling of rats followed by exposure to the elevated plus-maze either increases (Andrews *et al.*, 1991) or does not alter (Brett and Pratt, 1991) [3H]flunitrazepam binding to benzodiazepine receptors. Since the data in both studies appear to be derived from a single radioligand concentration, we have sought to resolve these discrepant results by determining the effect of repeated handling and elevated plus-maze exposure on [3H]flunitrazepam binding using full Scatchard analysis.

Male Sprague Dawley rats (70 - 90g at the start of the test; Charles River) were handled (as if to be dosed) daily for 28 days prior to placing in the centre of an elevated plus-maze 24h after the last handling; control rats were unhandled for 28 days apart from occasional routine animal care. Handled and unhandled rats were either exposed (10 min test) or not exposed to the elevated plus-maze (Luscombe *et al.*, 1990). The animals were then immediately killed, frontal cortex dissected and [3H]flunitrazepam (0.25 - 16 nM) binding, using 2 µM clonazepam to define non-specific binding, carried out essentially as described by Horton *et al.* (1982).

Handling for 28 days increased the open:closed ratio for arm entries (table 1), due to both an increased number of open arm entries (unhandled = 0.3 ± 0.2 entries, handled = 3.3 ± 0.9 entries; $p<0.01$, Student's *t*-test) and a decreased number of closed arm entries (unhandled = 15.3 ± 0.7 entries, handled = 6.0 ± 1.2 entries; $p<0.001$, Student's *t*-test); the total number of arm entries was decreased by handling (table 1). These changes were not accompanied by an alteration in either the affinity (Kd) or number (Bmax) of [3H]flunitrazepam binding sites, binding parameters which were also unaffected by exposure to the elevated plus-maze.

Table 1 The effects of handling and/or exposure to the elevated plus-maze on plus-maze performance and [3H]flunitrazepam binding

	Open:closed	Total	Rats not tested on maze		Rats tested on maze	
Pretreatment	arm entry ratio	arm entries	Kd	Bmax	Kd	Bmax
Unhandled	0.02 ± 0.01	15.7 ± 0.8	3.02 ± 0.16	2.26 ± 0.16	2.87 ± 0.22	2.29 ± 0.20
Handled	0.48 ± 0.13**	9.3 ± 1.9*	2.75 ± 0.16	2.31 ± 0.14	2.72 ± 0.17	2.55 ± 0.18

Kd (nM) or Bmax (pmol/mg protein) or arm entry values are mean ± s.e. mean for 6 determinations.
**$p<0.01$; *$p<0.05$ vs Unhandled (Student's *t*-test).

In conclusion, this study has shown that repeated handling altered the response of rats to the elevated plus-maze without altering the binding of [3H]flunitrazepam to rat frontal cortex. This is in agreement with the observations of Brett and Pratt (1991), but does not confirm the findings of Andrews *et al.* (1991).

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464P THE PHARMACOLOGICAL CHARACTERISATION OF RESPONSES TO THE BENZODIAZEPINE RECEPTOR LIGAND RU34347 IN THE RAT CEREBELLUM *IN VITRO*

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The imidazopyrimidine derivative RU34000 has been demonstrated to act as an inverse agonist at the GABA/benzodiazepine receptor complex *in vivo* (Gardner, 1989). The relatively soluble methane sulphonate salt RU34347 has been shown to act as an inverse agonist at higher concentrations (1-10µM) in the stimulus evoked inhibition of cerebellar Purkinje cells but as an apparent agonist at concentrations much below the IC_{50} for displacing [^{3}H]-flunitrazepam binding (Bagust *et al.*, 1990). This leaves the site of action of the drug unresolved. The purpose of this investigation was to further elucidate the mode of action of low concentrations of RU34347.

Extracellular recordings were obtained from single cerebellar Purkinje cells as previously described (Hussain *et al.*, 1990). 10nM RU34347 was shown to reduce the inhibition of the spontaneous firing rate (SFR) produced by 60-80 second pulses of 0.1mM GABA (17.67 ± 3.17% to 8.97 ± 3.02%, n=6, $p<0.01$), but also had a direct inhibitory action on the SFR (19.00 ± 4.41%, n=6). Further, the direct inhibitory action of 10nM RU34347 could be antagonised by 1µM flumazenil (30.06 ± 6.95% reduced to 17.7 ± 4.89%, n=5, $p<0.05$) and also by 1µM bicuculline (20.06 ± 3.35% reduced to 7.38 ± 2.56%, n=8, $p<0.01$). A dose-response relationship for RU34347 was found to be inverted (1nM - 12.71 ± 3.04%, n=9; 10nM - 10.67 ± 3.25%, n=7 and 100nM - 5.20 ± 3.09%, n=5).

These results are consistent with RU34347 acting as an inverse agonist against exogenously applied GABA, at a concentration well below the IC_{50} value of 980nM (Gardner, 1989). Antagonism of the decrease in SFR induced by 10nM RU34347 by 1µM flumazenil and 1µM bicuculline suggest that the compound is acting at central benzodiazepine receptors, and is also producing effects involving the action GABA. Together, these results suggest that RU34347 may be acting at a receptor other than the classical post-synaptic GABA/benzodiazepine complex in inducing the agonist-like effect at low concentrations. One possibility is that RU34347 acts as an inverse agonist on presynaptic benzodiazepine receptors leading to an enhanced release of GABA. The effects on firing rate would then be a balance of this presynaptic and direct postsynaptic effects. This possibility is under investigation.

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465P 2-OH-SACLOFEN AFFECTS BOTH EARLY AND LATE INHIBITION IN THE RAT HIPPOCAMPUS

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The actions of the GABA B antagonist 2-hydroxy-saclofen (2-OH-saclofen; Kerr et al.1988) in area CA1 of the rat hippocampal slice were investigated. Extracellular field potential recordings of population spikes, from stratum pyramidale, on response to orthodromic stimulation of the Schaffer collateral/commisural afferents, were used to investigate the GABAergic inhibition mechanisms present. A paired pulse protocol with interpulse intervals (IPI's) of between 10-500ms was employed to elicit inhibition. The stimulus was routinely set such that the conditioning response amplitude was half maximal. Response pairs for controls (n=17) revealed 'early', GABA A mediated, inhibition at short IPI's of 10-30ms and 'late', GABA B mediated, inhibition at longer IPI's of 200-400ms. Phaclofen (1mM) removed the late inhibition at 200-400ms but not the early, and had no effect on the conditioning response (n=8). However, 2-OH-saclofen (200μM) significantly attenuated the conditioning response amplitude (n=9; paired Student t-test <0.01) and the early (10-30ms) as well as the late (200-400ms) inhibition, measured as the increase in the percentage ratio of test/conditioning response amplitudes (T/C%), compared to control.

	Conditioning resp. (mV)		Test response (mV) at 20mS IPI					Test response (mV) at 300mS IPI				
	mean	±sem	mean	±sem	T/C%	±sem	sig.	mean	±sem	T/C%	±sem	sig.
Control	5.21	0.17	1.63	0.38	37	8		4.57	0.40	88	3	
2-OH-saclofen	3.34	0.20	3.05	0.37	93	12	<0.01	3.29	0.54	105	7	<0.02
wash-out	4.70	0.16	2.49	0.47	51	9		4.05	0.53	87	6	

The reduction of both phases of inhibition appeared to be independent of the reduction in the conditioning response, as judged from similar decreases in conditioning response amplitude produced by lowering the stimulus intensity. These results are consistent with the antagonist actions of phaclofen and 2-OH-saclofen on GABA B receptors (Harrison et al 1990), but 2-OH-saclofen actions to reduce early inhibition resemble those of the GABA B agonist baclofen, and may be caused by a partial agonist effect at GABA B receptors.

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466P VIGABATRIN AND ETHANOLAMINE-O-SULPHATE HAVE PERIPHERAL AS WELL AS CENTRAL EFFECTS ON THE GABAergic SYSTEM OF THE RAT

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Vigabatrin (GVG) and ethanolamine-O-sulphate (EOS) are inhibitors of GABA-Transaminase (GABA-T) the enzyme responsible for the catabolism of GABA. Both elevate brain GABA content and possess anticonvulsant effects (Jung *et al.*, 1977; Fletcher and Fowler, 1980). Neither GABA-T or glutamic acid decarboxylase (GAD) are located solely in the CNS (Erdo and Kiss, 1986) but information on GABA-T inhibition in the periphery is scarce even though (a) GVG is used clinically for the treatment of drug resistant refractory epilepsy normally following a chronic dosing regime and (b) both these drugs cross the blood brain barrier poorly.

We have examined the effects of these drugs following chronic (8 day) oral administration, on the levels of GABA-T and GAD in liver, kidney and brain and on GABA content of kidney, liver, brain and plasma on order to investigate any peripheral effects of these centrally acting drugs.

3 groups of 6 male Wistar rats (259 ± 16.5g) received in their drinking water: vehicle alone (1g/l sucrose), 3g/l GVG or 3g/l EOS for 8 days. Following sacrifice tissue samples and plasma were rapidly removed and frozen for subsequent analysis of GABA-T, GAD and GABA content (HPLC: precolumn ortho-pthaldialdehyde/2-mercaptoethanol derivatisation, elution with phosphate/methanol buffer gradient and subsequent fluorescence detection, Lindroth and Mopper, 1979).

As expected, inhibition of brain GABA-T (40-50%) leads to an increase in brain GABA levels (135-180% of control) and a decrease in GAD (30-50%) activity (as demonstrated by Fletcher and Fowler, 1980 and Jung *et al.*, 1977). This is mirrored in the liver (with a GABA-T inhibition of over 90%, GAD: 20-40%, GABA: 224-900% of control) plasma also shows a rise in GABA (to 175-343%) The kidney seems to have a different effect with >90% GABA-T inhibition: the GABA levels are reduced to 17-33% of controls, a decrease in GAD activity is also observed (30-65%).

The actual function of GABA-T in the periphery is unclear; we have shown that chronic treatment with GABA-T inhibitors leads to a >90% reduction in its peripheral activity. It is not known whether this is clinically important as few non-CNS side effects have been documented, however the severe inhibition obtained with the chronic dosing schedules currently employed, administering up to 4g daily could lead to problems of a non-CNS nature.

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467P EFFECTS OF BICUCULLINE AND NALOXONE INJECTIONS IN THE GLOBUS PALLIDUS OF THE RESERPINISED RAT

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It has previously been suggested that the neural mechanisms underlying parkinsonism involve an overactive striato-pallidal pathway (Pan et al.,1985). Striatal projections to the globus pallidus use GABA and enkephalin as co-transmitters (Haber and Elde,1981). This study tested the hypothesis that increased activity of these GABAergic/ enkephalinergic inputs to the pallidum mediates the symptoms of parkinsonism. Intracerebral microinjection techniques were employed to administer the GABAergic and enkephalinergic antagonists, bicuculline and naloxone, into the globus pallidus of the reserpinised rat model of parkinsonism.

Under general anaesthesia (60mg/kg pentobarbitone,i.p.), guide cannulae were implanted stereotaxically dorsal to the globus pallidus of male Sprague-Dawley rats. A minimum period of 72 hours recovery was allowed before induction of parkinsonism by an injection of reserpine (5mg/kg s.c.). 16-36 hours after reserpine administration compounds were injected into the globus pallidus. An injection needle was inserted down the guide cannula, the animals placed into an open field arena and injected unilaterally with either bicuculline, naloxone, saline or a combination of bicuculline and naloxone (injection volumes were 0.5 μl). Locomotor scores, related to distance moved by the forelimb ipsilateral to the injection site, were attained as described previously (Brotchie et al.,1991).

Parkinsonism, characterised by akinesia, rigidity and tremor, was seen prior to intrapallidal drug administration. Injections of bicuculline in the globus pallidus resulted in a marked alleviation of akinesia. The EC_{50} for this anti-parkinsonian effects was 393 fmol. This gave a mean locomotor score of 128.3 ($\pm$ 14.5, n=6) which was significantly different ($p<0.01$) from the saline injections, mean locomotor score 4.2 ($\pm$ 1.9, n=5). Naloxone injections (50nmol) had no significant effect on reversing akinesia compared with saline. Surprisingly, a co-injection of bicuculline and naloxone resulted in an attenuated anti-parkinsonian effect of bicuculline (70% reduction in locomotion, naloxone (50nmol) and bicuculline (393 fmol) vs bicuculline alone (393 fmol), $p<0.01$). The dose-response curve for the anti-parkinsonian effects of bicuculline was shifted to the right in the presence of naloxone (50nmol), the EC_{50} being 1.01 pmol.

Parkinsonism can be alleviated by the injection of bicuculline into the globus pallidus of the catecholamine-depleted rat. This action probably reflects antagonism of an abnormally overactive GABA input from the striatum. However, antagonism of the similarly overactive enkephalin input, did not alleviate parkinsonism. The naloxone-mediated attenuation of responses to bicuculline may involve antagonism of an enkephalinergic action that results in decreased GABA-mediated responses. Elucidation of peptidergic modulation of GABA transmission in the globus pallidus may prove critical for obtaining a better understanding of basal ganglia function in the control of voluntary movement and in the mediation of movement disorders.

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468P FAILURE OF NALTREXONE AND MORPHINE TO REVERSE THE EFFECTS OF FASTING ON STRIATAL DOPAMINE AND 5-HT METABOLISM IN MICE

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Fasting is known to increase the concentrations of the metabolites of dopamine (DA) and 5-hydroxytryptamine (5-HT) in the mouse striatum (Fuenmayor, 1979). In mice and rats morphine (MO) and other opioids produce similar effects on amine metabolism, which are antagonized by naloxone or naltrexone (NTX) (Ahtee & Attila, 1987). In the present experiments we studied, by using the opioid agonist MO and antagonist NTX, whether the endogenous opioid systems of mice are involved in the changes of striatal monoaminergic systems induced by fasting.

One group of the mice had free access to food and water while food was removed from the other group. After 24 h both groups were treated with NTX (10 mg/kg s.c.), MO (10 mg/kg s.c.) or saline. 1 h after the treatment the mice were decapitated. The striatal DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were measured using HPLC-ED.

The 24 h fast increased the concentrations of the DA metabolites HVA and DOPAC (by 29 and 17%) as well as 5-HT metabolite 5-HIAA (by 37%). MO increased the DOPAC, HVA and 5-HIAA concentrations by 45, 33 and 25%, while NTX did not induce any effect on the fed mice. NTX clearly potentiated the increasing effect of fasting on the DOPAC and HVA as well as on the 5-HIAA concentrations (29, 19 and 35% increase by NTX). Also MO potentiated the increase of DOPAC and HVA (39 and 16% increase by MO) and less clearly that of the 5-HIAA concentration induced by fasting.

These results suggest that in mice, in contrast to rats (De Montis *et al.*, 1978), the endogenous opioid systems are not to directly involved in the enhanced activity of striatal DA- and 5-HT-ergic systems induced by fasting. In this study exogenous MO enhanced the DA and 5-HT metabolism both in the fasted and fed mice while NTX had no effect on the fed mice and did not antagonize but potentiated the increasing effect of fasting on the DA and 5-HT synthesis and metabolism.

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469P ACTIONS OF THE NEUROACTIVE PEPTIDE, APGWamide, ON CENTRAL NEURONS OF *HELIX ASPERSA*

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APGWamide, H-Ala-Pro-Gly-Trp-NH_2, was isolated from the ganglia of a prosobranch mollusc, *Fusinus ferrugineus* and found to have presynaptic modulatory actions on contractions in molluscan muscles (Kuroki *et al.*, 1990). However, the neuronal mechanisms of APGWamide on the central nervous system of molluscs is unknown. The aim of this study was to elucidate the mode of action of APGWamide on the central neurons of *Helix aspersa*.

Experiments were performed on identified neurons from isolated suboesophageal ganglia of *Helix aspersa* using conventional intracellular recording (Kerkut *et al.*, 1975). In F1 neuron at concentrations between 0.1-5μM, APGWamide reversibly inhibited the amplitude of evoked-IPSPs. For example, 1μM APGWamide reduced IPSPs to 28.8 ± 3.0% of control (n=3, ± s.e. mean). These IPSPs are dopaminergic and are due to an increase in potassium conductance (Kerkut *et al.*, 1969). APGWamide had no direct effect on membrane potential, cell firing rate, or dopamine-induced responses. These results indicate that the actions of APGWamide in F1 neurons are on presynaptic sites.

In contrast APGWamide had direct effects on F2 neurons. At concentrations between 0.5-10 μM, APGWamide inhibited spontaneous spike activity and hyperpolarized the membrane potential of F2 neurons in a dose-dependent manner. In K^+ free solution, the inhibitory effects of APGWamide were potentiated, while they were gradually reduced by increasing external K^+concentration (8-16 mM). For example, in K^+ free solution, the control responses induced by 5μM APGWamide increased to 142.9 ± 5.8% (n=4 ±s.e.mean), in 8mM K^+ solution, control responses decreased to 53.6 ± 5.3% (n=4, ± s.e.mean) and in 12mM K^+ solution, responses were reduced to 22.1 ± 4.4% of control (n=4, ± s.e.mean) Applications of potassium channel blockers, either tetraethylammonium (10 mM) or 4-aminopyridine (500 μM), only partially prevented the inhibitory effects of APGWamide on F2 neurons. APGWamide (5-10 μM) only slightly increased membrane conductance. This evidence indicates that at least part of the actions of APGWamide are due to an increase in potassium permeability on F2 neurons. Further studies are required to determine the type of potassium channel involved.

In this study we conclude that APGWamide can exert both presynaptic and postsynaptic effects on central neurons of *Helix aspersa*. The postsynaptic effect is associated with an increase in potassium permeability.

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470P THE INVOLVEMENT OF α-ADRENOCEPTORS IN THE POTENTIATION OF OPIOID ANTINOCICEPTION BY SYMPATHOMIMETICS IN MICE

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Many codeine-containing preparations are freely available over the counter in most countries and increasing non-medical use of such preparation has been reported. In Hong Kong there is an increasing abuse of proprietary formulations of "cough remedies" containing ephedrine and codeine (Chan et al., 1990). It has been reported previously in the acute setting ephedrine and phenylpropanolamine (PPA) potentiate the antinociceptive activities of both morphine and codeine (Dambisya et al., 1990). The mechanism by which these sympathomimetics modify opioid action has so far been largely speculative. The present study attempts to interpret these interactions in terms of adrenoceptor activation.

Male ICR mice weighing between 30-35g were used. Antinociceptive effect was measured by the tail flick method. The peak effects of codeine and morphine occurred at 15 min and 30 min, respectively, after subcutaneous (sc) administration. Ephedrine and PPA were administered intraperitoneally (ip) 10 min prior to the opioid. The various adrenoceptor antagonists were given sc 10 min before the sympathomimetics. The data for the mean tail flick reaction time were analyzed and compared by means of the unpaired Student's t-test.

Ephedrine and PPA (10mg kg^{-1}, ip) pretreatment potentiated the antinociceptive effects of morphine 5mg kg^{-1}, sc (8.44 ± 0.54s and 7.88 ± 0.41s vs 6.43 ± 0.54s, $P<0.05$) and codeine 60mg kg^{-1}, sc (8.24 ± 0.49s and 8.35 ± 0.48s vs 6.80 ± 0.51s, $P<0.05$). Prior administration of propranolol (6mg kg^{-1}, sc) 10 min before the sympathomimetics had no effect on this action. Phentolamine (2mg kg^{-1}, sc), on the other hand, abolished the enhancing effects of ephedrine and PPA on opioid antinociception. The tail flick reaction times for morphine were decreased from 8.44 ± 0.54s to 6.86 ± 0.54s ($P<0.05$) for the ephedrine group and from 7.88 ± 0.41s to 6.48 ± 0.54s ($P<0.05$) for the PPA group respectively. Prazosin (2mg kg^{-1}, sc) pretreatment did not significantly affect the potentiation of opioid antinociception by ephedrine and PPA, while yohimbine (4mg kg^{-1}, sc) effectively antagonized this enhancing effect. None of the adrenoceptor antagonists had any effect on the tail-flick reaction time on their own in the doses used, and neither did they affect opioid antinociceptive responses. The doses of the adrenoceptor antagonists used in the present study has been shown by others to selectively block the respective receptors (Doherty and Hancock, 1983). It is concluded that ephedrine and PPA potentiate the antinociceptive effects of morphine and codeine most probably through an action on α_2-adrenoceptors.

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471P NEUROKININ RECEPTOR SUBTYPES MEDIATING THE DEPOLARISATION OF NEURONES IN RAT AND GUINEA-PIG SUPERIOR CERVICAL GANGLIA ARE DIFFERENT

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At least three types of neurokinin receptors are present on neurones (Iversen *et al.*,1990). Here we provide evidence for heterogeneous populations of neurokinin receptors in both rat and guinea-pig superior cervical ganglia and furthermore our data suggest that the proportions of these receptor subtypes differ between the two species.

DC recordings were made from isolated rat and guinea-pig superior cervical ganglia using grease gap methodology in the presence of tetrodotoxin (0.1 μM). Concentration-response curves were fitted using a single-site logistic model for a range of neurokinin receptor agonists with all data normalised to the depolarisations caused by 1 μM eledoisin (Max %). Data is the mean ± SEM (n = 3 to 9).

	RAT		GUINEA-PIG	
	EC50 (nM)	Max (%)	EC50 (nM)	Max (%)
SENKTIDE	4 ± 2	27 ± 3	37 ± 14	274 ± 24
SUBSTANCE P (SP)	12 ± 3	72 ± 4	Biphasic (25% @ 1 μM)	
ELEDOISIN	33 ± 9	103 ± 8	1918 ± 97	273 ± 5
NEUROKININ B (NKB)	118 ± 65	132 ± 18	1236 ± 545	259 ± 20
NEUROKININ A (NKA)	268 ± 35	140 ± 5	Biphasic (26% @ 1 μM)	

In rat ganglia the greater maximum depolarisation of SP relative to senktide, and the rank order of potency of SP, NKA, and NKB, suggest that the predominant receptor is an NK1 subtype. In contrast, in guinea-pig ganglia, senktide has a greater maximum than SP and the predominant subtype appears to be NK3. The small but potent effects of senktide and SP, in rat and guinea-pig ganglia respectively, suggest that heterogeneous populations of neurokinin receptors are present in both these species.

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472P EFFECTS OF ANTAGONISTS SELECTIVE FOR NEUROKININ-1 AND NEUROKININ-2 RECEPTORS ON SOMATOSENSORY RESPONSES OF RAT DORSAL HORN NEURONES

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Although for many years substance P (SP) has been a candidate for the transmitter of polymodal nociceptive somatosensory afferents, there is now considerable evidence that neurokinin A (NKA) or related peptides may be important (Fleetwood-Walker *et al*, 1990). Neurokinin A (NKA) and related structures show selectivity for NK-2 receptors and there is increasing evidence for the presence of not only NK-1, but also NK-2 and NK-3 receptors in superficial dorsal horn (Yashpal *et al*, 1990). In the present study, the effects of ionophoretically-applied L-668169 and L-659874 (highly-selective antagonists at NK-1 and NK-2 receptors respectively; McKnight *et al*, 1988) were investigated on the somatosensory responses of neurones in rat dorsal horn. Extracellular recordings were made from lamina IV/V neurones in segments T12 - L4 of chloralose/urethane-anaesthetised rats, as described previously (Hope *et al*, 1990). Neuronal responses to repeated peripheral stimuli were recorded using the central barrel of a glass microelectrode, with ionophoresis of drugs (as 0.15 mM, pH 4.5 - 5, aqueous solutions with 0.3% dimethylformamide) from the side barrels.

In none out of 6 neurones tested with L-668169 (at 20 nA for 6 min), was there any marked inhibition of responses to noxious heat (84 ± 6% of controls) noxious pinch (104 ± 9% of controls) or spontaneous activity (94 ± 6% of controls), (mean ± s.e. mean in each case). However, in 5 out of 6 cases there was clear enhancement of responses to innocuous brush or tapping, reaching a mean of 143 ± 7% of control values (n = 5). In one neurone, spontaneous activity and sensory responses were all increased. In contrast, the NK-2 antagonist L-659874 selectively inhibited thermal nociceptive responses in all except one neurone tested. In the other 6 cases, thermal nociceptive responses were reduced to 43 ± 7% of controls by L-659874 at 10 nA for 3 min (n = 3) and to 23 ± 10% of controls by 20 nA for 3 to 6 min (n = 3). Corresponding mean responses to pinch, brush/tap and spontaneous activity remained within 10% of control values. These results are entirely in accord with those on antidromically-identified neurones in the cat (Fleetwood-Walker *et al*, 1990) indicating that NK-2 receptors serve a crucial role in the transmission of thermal nociceptive inputs. It is possible that NK-1 receptors also subserve a role in nociception; but at sites not well accessed here. A robust influence of NK-1 receptors in the regulation of non-nociceptive responses was also apparent (as observed in previous experiments on cat dorsal horn neurones; Fleetwood-Walker *et al*, 1990). Furthermore, the direct effects of L-668169 observed here suggest that a tonic influence of an endogenous NK-1 agonist such as SP may be present during our testing protocol.

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473P CHARACTERIZATION OF NEUROKININ RECEPTORS IN THE MOUSE WHICH MEDIATE ANTIDIPSOGENIC ACTIVITY

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The neurokinins (NK), substance P (SP), NKA and NKB have all been found to exhibit antidipsogenic activity following intracerebroventricular (i.c.v.) administration to rodents (Fitzsimons and Evered, 1978; Massi et al., 1988). In the present study we have investigated the effects of potent, selective and stable agents, on the drinking response of water-deprived mice to elucidate the NK receptors involved in this effect. The agonists used in the present study were GR73632 (NK_1), GR64349 (NK_2) (Hagan et al., 1991a) and senktide (NK_3). The antagonists used were GR82334 (NK_1;Hagan et al., 1991b) and L-659,877 (NK_2;Maguire et al., 1989).

Male albino mice (CRH:12-16g; Glaxo, Ware) had access to water withdrawn 16h prior to drug administration on the test day. Mice were placed individually in separate boxes (30x13x13cm), to which a burette containing tap water was attached and the level recorded over a 20min period. In a separate series of studies the behavioural profiles of each NK agonist, following i.c.v. administration were investigated. Drugs and their vehicles were injected using a procedure described previously (Brittain and Handley, 1967). Agonists when given alone, or in combination with antagonists, were administered in a 3.0µl volume over a 1.0min period. Verification of the injection site was established prior to statistical analysis.

Water deprivation in mice elicited an increase in water consumption as compared with control animals; water deprived animals:305±55µl $20min^{-1}$;control mice:5±5µl $20min^{-1}$. The water deprivation-induced dipsogenic response was inhibited dose-dependently by all 3 NK agonists. Hence a second study was undertaken to investigate the possibility of behavioural competition. Doses of GR73632 (100 & 300pmol) which reduced water consumption also increased locomotor activity and scratching. A dose of senktide (10pmol) which decreased drinking also induced a 5-HT-like syndrome which included wet-dog shakes, flattened body posture, forepaw treading, tail rattle and hind limb splaying. The NK_2 agonist, GR64349 (1-3000pmol) appeared to induce a genuine antidipsogenic response as this effect was not compromised by any other behavioural effects and was blocked by the NK_2 antagonist, L-659,877 (10nmol), but not by the NK_1 antagonist, GR82334 (0.1 or 1.0nmol).

These results support an involvement of NK_2 receptors in the antidipsogenic effects of the NKs. Furthermore, as the NK_2 antagonist had no effect on the drinking response of the water replete or deprived mice it may be inferred that there is little or no endogenous NK tone in systems which regulate water consumption.

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474P DETECTION OF CCK RECEPTOR SUBTYPES IN RAT BRAIN USING NOVEL DIPEPTOID CCK ANTAGONISTS

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Cholecystokinin (CCK) receptors have been divided into two major subtypes, (CCK-A and CCK-B), originally classified according to their affinity for various CCK fragments. Recent studies have shown that although the majority of CCK receptors in mammalian brain are of the CCK-B type, CCK-A receptors are also present in the CNS (Hill *et al.*,1987). In the present study we have used three novel "dipeptoid" compounds (CI-988, PD 135158 and PD 136450; Horwell *et al.*,1990; Hughes *et al.*,1990), possessing nanomolar affinity and high selectivity for CCK-B receptors, to expand on these observations and further examine CCK receptor distribution in rat brain using *in vitro* receptor autoradiography.

Sections (10µm) were preincubated in assay buffer (composition in mM: HEPES 20, NaCl 130, KCl 4.7, $MgCl_2$ 5 and EGTA 1; pH 7.4 at 21°C) for 10min. Adjacent sections were then incubated in assay buffer containing 0.25mg/ml bacitracin and 50 pM [^{125}I]-Bolton Hunter-CCK8 in the absence and presence of varying concentrations of test compounds for 120 min at room temperature. Non-specific binding was defined by 1µM sulphated CCK8. After washing (4 X 6min in ice-cold buffer) sections were dried and exposed to Amersham Hyperfilm for 10-14 days. Autoradiographs were analysed using the Quantimet 920 image analysis system and pIC50 values determined for each compound.

In keeping with earlier studies (Hill *et al.*, 1987), high densities of CCK binding sites were observed in cingulate cortex (CCX), ventro- and dorsomedial hypothalamus (VMH and DMH respectively), habenular (HBN) and interpeduncular nucleus (IPN). CCK8S (1µM) uniformly displaced [^{125}I]-BH-CCK8 binding from all areas. CI-988, PD 135158, PD 136450 and L-365,260 (Chang et al, 1989) selectively displaced [^{125}I]-BH-CCK8 binding to cingulate cortex, VMH and amygdala (AMY) in a concentration dependent manner, with pIC50 values ranging from 7.4 (L-365,260 in VMH) to 8.3 (PD CI-988 in CCX). CI-988 appeared to inhibit all [^{125}I]-BH-CCK in the VMH even though recent studies have revealed the presence of a low density of CCK-A receptors within the VMH (Pinnock et al, this meeting). The peptoids were less effective at inhibiting [^{125}I]-BH-CCK8 in the DMH, HBN and IPN whereas MK-329 was a potent inhibitor of binding (pIC50 = 9.0) suggesting that these nuclei contained predominantly CCK-A sites.

These results confirm and expand previous studies showing the presence of CCK-A receptors in rat brain.

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475P AN AUTOMATIC TELEMETRY SYSTEM FOR COLLECTION OF SMALL MAMMAL BODY TEMPERATURE DATA

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This system links temperature-sensitive AM transmitters (Model X, MiniMitter Inc., USA) with low-cost hardware and software developed from a design of G. Titmus (unpublished) at the Polytechnic of East London for studies on mice. Each transmitter, embedded in paraffin wax (total weight c.1.5g), is implanted aseptically in the peritoneal cavity of the experimental animal. (Before this, transmission pulses from each transmitter are calibrated, timing inter-pulse intervals v. temperature.) Subsequent use is limited by battery life (3-4 weeks minimum). Sensitivity to temperature change within animals is of the order of 0.1-0.2°C. Software (for a BBC computer OS 1.20) allows many choices from continuous monitoring of a single animal, to cyclic monitoring (every 6 min or more) of 12 animals (in separate cages placed on individual aerial mats). Body temperature is computed (via a BASIC progam) from inter-pulse intervals in the 30 second period monitoring a given aerial input in each cycle. Low and high inter-pulse values (radio interference or silence) are edited out prior to calculating mean interval. The number of valid pulses gives a reliability index for each estimate. The system tackles the same problems as that of Ruf & Heldmaier (1987), but is cheaper (a single radio switching between aerials), and relies more on software than hardware to clean up occasional radio interference.

Body temperatures in small mammals (with high relative metabolic rates and low mass) are labile. Stress-inducing events (handling, injections, operations, cage cleaning etc.) are soon detectable as changes in body temperature. Telemetry has the clear advantage of avoiding the repeated handling associated with invasive measurement of colonic temperature. Procedure-induced temperature changes can be reduced to the minimum required for application of a pharmacological treatment, rather than for later measurement of its effect. The system also allows automatic monitoring of diurnal temperature profiles of undisturbed animals for days before and after experimental treatment. Huge databases can be collected for each animal. A statistical package (MINITAB) is used routinely (i) to analyse acute within-experiment responses (Sturman *et al.*, 1991), (ii) to detect trends in underlying diurnal rhythms (Jakobson & Andrews, 1989), (iii) to assess the impact of standard procedures on body temperature.

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476P COMPUTER-SIMULATED PHARMACOLOGICAL PREPARATIONS FOR TEACHING NEUROMUSCULAR PHARMACOLOGY

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Various computer simulated pharmacological preparations are available (Hughes, 1987) and can be used in several ways as an adjunct to normal laboratory classes. When laboratory experiments fail, a simulation can provide a record (on screen or printer) which can be measured and written up. Simulations allow students to perform more extensive experiments than in the real laboratory since data is provided quickly and without time-consuming laboratory preparation. Furthermore, sharp time constraints often apply in the real laboratory; with simulations students can use learn-by-discovery methods without wasting animals or time. In this context simulations are particularly effective in allowing students to learn experimental design.

The simulations described here are IBM-compatible programs which allow students to use the three classical preparations (frog rectus; phrenic nerve-diaphragm; sciatic nerve-gastrocnemius muscle in vivo) to study neuromuscular pharmacology. The simulations are menu driven and impose few constraints; thus the user must choose the drug, the concentration (dose) and has control over the sequence of events. The simulations are therefore very flexible. Teachers can set a schedule of drug administrations for the student to follow; alternatively a question can set and the student left to design their own experiment. The level and complexity of the task is controlled by the teacher who can ensure suitability for the particular students under instruction.

Competitive and depolarising neuromuscular blockers, anticholinesterase drugs and nicotinic agonists are available as, when appropriate, are potassium, 4-aminopyridine, triethylcholine, choline, dantrolene, lignocaine, atropine and others. The simulation will provide an unknown neuromuscular blocker the properties of which can be discovered by experiment. When appropriate electrical stimulation can be applied at 0.5, 0.05 Hz or as a short tetanus. Once administered in vivo drugs wear off with an appropriate half-life. The user can swap from preparation to preparation as required (for example to characterise an unknown). On leaving the simulation completely however the characteristics of the unknown are revealed. If the program is run again a different unknown will be provided. The simulations are popular with students and in some circumstances, where appropriate teaching objectives are defined (Hughes, 1991), can be used as an alternative to animal experiments.

Hughes, I. E. (1991) ATLA, 19, 271-276.
Hughes, I.E. (1987) Br. J. Pharmac., 90, 290P

477P A NOVEL HYPOTHESIS FOR THE DIAPEDESIS OF NEUTROPHILS: A ROLE FOR KININS

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Neutrophils attracted by cytokines (interleukin-1, tumour necrosis factor) in the early phases of inflammation, marginate and adhere to endothelial cells. Once neutrophils have adhered to the endothelial cells the next phase is the opening of gap junctions between the cells. The mechanisms involved in this phase are not known.

Neutrophils were isolated from leucocyte enriched plasma over Ficoll-Hypaque gradients. Air-dried smears and pellets of neutrophils were processed for immunocytochemistry and visualisation by light, confocal and electron microscopy. Both fixed and non-fixed cells were used for confocal microscopy. The first antibody was specific for each of the antigens and the second either rabbit or mouse fluorescein labelled (Fab_2) IgG. Cell membrane F_C receptors were blocked with non-immune goat serum, in order to prevent non-specific binding of the antibodies.

Evidence will be reported for the presence of a tissue kallikrein (TK) in granules (Figueroa et al, 1989) and the localization of L-kininogen (LK) and H-kininogen (HK) (Figueroa et al, unpublished) and plasma pre-kallikrein on the external surface of the neutrophil memebrane. Our findings raise important questions with regard to the role of neutral proteases, like kallikreins and their substrates kininogens, in the normal adhesion process and the subsequent migration of neutrophils through gap junctions. A novel hypothesis would be the local release of kinins on the surface of the neutrophil membrane to open a corridor between endothelial cells and facilitate migration of neutrophils into the interstitial tissue space. Such a functional role for kinins merits further study.

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Figueroa, C.D., MacIver, A.G. & Bhoola, K.D. (1989) Br. J. Haematol. 72, 321-328.
Figueroa, C.D., Henderson, Lydia, Kaufman, J., De la Cadena, R.A., Colman, R.W. & Müller-Esterl, W. Blood, submitted.

ORAL COMMUNICATIONS

In oral communications with more than one author, the first author is the one who intended to present the work

1P **Guild SB, Jenkinson S & Muir TC** Altered inositol phosphate metabolism in tail arteries in spontaneously hypertensive rats

2P **Dalziel HH, Thornbury KD, Ward SM, Carl A, Westfall DP & Sanders KM** Involvement of nitric oxide in inhibitory junction potentials in canine proximal colon

3P **Ward SM, Dalziel HH, Thornbury KD, Westfall DP & Sanders KM** Non-adrenergic, non-cholinergic inhibition and rebound excitation in canine proximal colon are mediated by nitric oxide

4P **Lefebvre RA, Hasrat J & Gobert A** Influence of N^G-nitro-L-arginine methyl ester on vagally-induced gastric relaxation in the rat

5P **Boyle FC, Craig JW & Muir TC** Nitric oxide in inhibitory transmission in the guinea-pig lower oesophageal sphincter

6P **Craig JW & Muir TC** Nitric oxide in inhibitory transmission in the guinea-pig internal anal sphincter

7P **Sheng H, Mitchell JA, Nakane M, Warner TD, Pollock JS, Förstermann U & Murad F** Characterisation of NO-synthase fron NANC nerve-containing tissues: rat anococcygeus and bovine retractor penis muscles

8P **Watson N, Maclagan J & Barnes PJ** Role of endogenous vasoactive intestinal polypeptide and nitric oxide in pre- and post-ganglionic cholinergic neurotransmission in guinea-pig trachea

9P **Whiting MV & Cambridge D** The role of endothelial-derived nitric oxide in modulating the renal vasoconstrictor response to 5-HT_1-like receptor agonists

10P **Gardiner SM, Kemp PA, Bennett T, Palmer RMJ & Moncada S** Sustained hypertension in Brattleboro rats during chronic oral ingestion of N^G-monomethyl-L-arginine

11P **Bennett T, Gardiner SM & Kemp PA** N^G-nitro-L-argninine methyl ester enhances the pressor effects of adrenaline in conscious rats

12P **Trezise DJ & Weston AH** EDRF release accounts for the depressant effect of the endothelium on contractions of rabbit isolated basilar artery to 5-HT

13P **Lawrence E & Brain SD** The effect of L-N^G-nitro arginine methyl ester (L-NAME) and endothelins (ET-1 and ET-3) on cutaneous blood flow in the diabetic rat

14P **Muscholl E & Habermeier-Muth A** Sympathetic modulation of acetylcholine release from rabbit atria evoked by vagus nerve stimulation: a non-adrenergic facilitation and a noradrenergic inhibition

15P **Clarkson PN, Growcott JW, Wilson C & Cox B** The effects of α-methyl 5-HT on the rabbit isolated right atrium

16P **Walker GB, Bruin J, Cottney JE, Shahid M & Spiers I & Nicholson CD** Differential effects *in vitro* of the enantiomers of Org 20494

17P **Shahid M, Bruin JC, Walker GB, Cottney JE & Nicholson CD** Positive inotropic, Ca^{2+}-sensitizing and cyclic nucleotide phosphodiesterase (PDE) isoenzyme inhibitory effects of Org 30029 in human atrial myocardium

18P **Weishaar RE, Wallace AM, Kirker ML, Ferraris VA & Sim MF** Comparative effects of flosequinan and milrinone on the contractile activity of cardiac muscle fibres from patients with and without heart failure

19P **Rees SA & Curtis MJ** Effects of UK-66914 on ischaemia-induced and reperfusion-induced arrhythmias in rat

20P **Wainwright CL & Parratt JR** The effects of CV1808, an A_2-adenosine receptor agonist, on ischaemic arrhythmias in anaesthetized pigs

21P **Saville VL, MacKenzie I, Carr MG & Kay PB** Glibenclamide, but not the Class III compound E-4031, prolongs ischaemic action potentials in guinea-pig papillary muscles

22P **Boachie-Ansah G & Kane KA** Modification by hyperkalaemia of the electrophysiological effects of isoprenaline on guinea-pig ventricular myocytes

23P **Ramsay GA & Terrar DA** Halothane actions on the cytosolic calcium transient during action potentials in vintricular cells isolated from guinea-pig heart

24P **Coker SJ, Edwards KM & Barnes CS** Zaprinast potentiates adenosine-induced bradycardia at doses that do not alter haemodynamic responses to sodium nitroprusside or salbutamol

25P **Sheridan RD, Patmore L & Spedding M** The novel neuroprotective agent, RS-87476, potently inhibits sodium currents in mouse neuroblastoma

26P **Faller D, O'Reilly C & Ryan MP** Drug-sensitivity of sodium uptake in human placental brush border membrane vesicles

27P **Watling KJ, Guard S, Howson W & Walton L** Inhibition of binding to tachykinin NK_2 receptor sites in rat, guinea-pig and human brain by the non-peptide substance P antagonist (±)CP-96,345

28P **Birch PJ, Rogers H & Harrison SM** Activity of a non-peptide NK_1 antagonist, racemic CP-96,345, at NK_1 receptors in the rat and mouse *in vivo*

29P **Hayward NJ & Woodruff GN** Neuroprotective effect of the κ-agonist, CI-977, in the gerbil model of global ischaemia

30P **Howat DW, Lisle HS & Higgs GA** The role of tumour necrosis factor and the leukocyte adhesion molecule CR3 in endotoxic shock

31P **Berman RS & Martin W** Homocysteine-induced endothelial barrier dysfunction assessed by an *in vitro* model

32P **Taylor PD, McCarthy AL, Thomas C & Poston L** Endothelial function and noradrenaline sensitivity in mesenteric resistance arteries of rats with streptozotocin-induced diabetes

33P **Dunne MJ, Chan SLF & Morgan NG** Block of ATP-regulated K^+ channels by α_2-adrenoceptor antagonists in insulin-secreting cells

34P **Harvey AL, Anderson AJ, Rowan EG, Marshall DL, Castaneda O & Karlsson E** Dendrotoxin-like activity isolated from sea-anemones

35P **McLaughlin DP, Beats B, Harrison PJ, Cairns NJ & Kerwin RW** Comparative second messenger binding and *in situ* hybridization studies of the medial temporal lobe in Alzheimer's disease

36P **Bristow DR, Arias-Montaño & Young JM** Sensitivity to lithium ions of agonist-induced inositol phosphate accumulation in HeLa cells

37P **Connick J, Hanlon G, France L, Fox PK & Nicholson CD** An investigation of the coupling of sigma binding sites to a guanine nucleotide binding (G) protein

38P **McClue SJ & Milligan G** The human α2-C10 adrenoceptor can interact with both G_i2 and G_i3 when expressed in Rat 1 fibroblasts

39P **Plevin R, Stewart A, Paul A & Wakelam MJO** Vasopressin-stimulated phosphatidylinositol 4,5-bisphosphate and phosphatidylcholine hydrolysis in A10 vascular smooth muscle cells

40P **Marker AJ, Galloway E & Bushfield M** The role of cyclic AMP in the control of hepatocyte proliferation by hepatocyte growth factor

41P **Hall JM, Field JL, Mitchell D & Morton IKM** Putative bradykinin BK_3 receptors in the guinea-pig taenia caeci and trachea

42P **Farmer SG, Wilkins DE & Meeker SN** Effects of bradykinin antagonists on antigen-induced pulmonary distress, airway hyperresponsiveness and eosinophilia in guinea-pigs chronically challenged with ovalbumin

43P **Barnes JC, Brown JD, Hawcock AB & Michel AD** Angiotensin II-induced contractions of the isolated preparation of rat uterus are mediated through the AT_1 receptor subtype

44P **Eglen RM, Michel AD, Levine WB & Whiting RL** Analysis of muscarinic receptors in guinea-pig oestrogen-dominated uterus, *in vitro*

45P **Patel M, Spraggs CF & Jordan CC** Comparisons of the selective CCK agonists A71378 and A72962 in guinea-pig ileum

46P **Ford APDW, Clarke DE, Eglen RM & Baxter GS** 5-HT stimulated adenylyl cyclase in rat oesophageal tunica muscularis mucosae (TMM) via non-classical 5-HT receptors

47P **Baxter GS & Clarke DE** Actions of benzimidazolone derivatives at putative 5-HT_4 receptors in rat oesophagus

48P **Whiting RL, Harris GC & Eglen RM** Characterization of muscarinic receptors in canine femoral vein *in vitro*

49P **Parkinson NA, Hughes AD & Sever PS** Sources of calcium mobilised by α-adrenoceptors in human subcutaneous resistance arteries

50P **Redfern WS & Clague RU** Effects of RS-15385-197 and other α_2-adrenoceptor antagonists on tail skin temperature in unanaesthetized rats

51P **Aidulis DM, Fuglsang A, Savage SAH & Pollock D** Drug-induced enhancement of contractions to clonidine in rat tail artery rings *in vitro*

52P **Hughes SR & Brain SD** The effect of a calcitonin gene-related peptide antagonist ($CGRP_{8\text{-}37}$) and indomethacin on blood flow induced by intradermal capsaicin

53P **McAuley MA & Westfall TC** Neuropeptide Y receptor subtypes: possible location and function in the rat mesenteric arterial bed

54P **Lawson K, Martin DJ, Zazzi-Sudriez E, Armstrong JM & Hicks PE** Endothelin-1 attenuates the vasodilator responses to BRL-38227 and Ro 31-6930, but not RP-49356 or nitrendipine, in the spontaneously hypertensive rat

55P **Millar C & Wilson WS** Comparison of the effects of vasodilator drugs on intraocular pressure and vascular relaxation

56P **Brock JA & Cunnane TC** Evidence that activation of α_2 adrenoceptors does not inhibit calcium entry into postganglionic sympathetic nerve terminals

57P **Searl T, Brock JA & Cunnane TC** Effects of clonidine on the action potential in cell bodies and nerve terminals of sympathetic neurones innervating the guinea-pig vas deferens

58P **MacDonald E, Laitinen K, Konstandi M & Tuomisto L** Tolerance develops to the neurochemical changes in rat brain after treatment for 10 days with the specific α_2-adrenoceptor antagonist, atipamazole

59P **Benwell MEM, Balfour DJK & Lucchi HM** The influence of calcium on extracellular dopamine levels in the nucleus accumbens

60P **Daly SA & Waddington JL** The new selective D_1 dopamine receptor agonist A-68930: psychopharmacological characterisation in relation to D_1:D_2 interactions

61P **Löschmann P-A, Kunow M, Schöllkopf K, Rettig KJ & Wachtel H** Contraversive turning induced by the dopamine D_1 agonist SKF 89 626 in unilaterally substantia nigra lesioned rats

62P **Biggs CS, Fowler LJ, Pearce BR & Whitton PS** The effect of sodium valproate on dopamine and 3,4-dihydroxyphenylacetic acid levels in rat hippocampal dialysates *in vivo*

63P **Banks RJA & Dourish CT** The angiotensin receptor antagonists DuP 733 and WL 19 block apomorphine-induced stereotypy in the rat

64P **O'Connor JJ & Kruk ZL** 5-HT_1-like and 5-HT_{1B} receptor ligands but not 5-HT_{1A} ligands modulate 5-HT release in the suprachiasmatic nucleus of the rat

65P **Anderson IK** Evidence to suggest that activation of forebrain 5-HT_1 receptors causes sympathoexcitation in anaesthetized rats

66P **Pangalos MN, Francis PT, Foster AC, Pearson RCA, Middlemiss DN & Bowen DM** 5-HT_{1A} and NMDA but not 5-HT_2 receptors are enriched on rat neocortical pyramidal neurones and selectively destroyed by intrastriatal volkensin

67P **Martin KF, Hannon SD, Phillips I & Heal DJ** Opposing roles for 5-HT_1 and 5-HT_3 receptors in controlling 5-HT release in rat hippocampus *in vivo*

68P **Malone HM, Peters JA & Lambert JJ** (+)-Tubocurarine and cocaine reveal species differences in the 5-HT receptors of rabbit, mouse and guinea-pig nodose ganglion neurones

69P **Wright IK, Upton N & Marsden CA** Effect of diazepam on extracellular 5-HT in the ventral hippocampus observed in rats on the X-maze model of anxiety using *in vivo* microdialysis

70P **Pasternack M, Bountra C, Voipio J & Kaila K** Effect of external and internal pH on GABA-activated chloride conductance and its desensitisation in crayfish muscle fibres

71P **Suman-Chauhan N, Hill DR & Woodruff GN** [^{3}H]-Gabapentin binds to a novel site in rat cortical synaptic plasma membranes

72P **Hill DR, Suman-Chauhan N & Woodruff GN** Autoradiographical detection of [^{3}H]-Gabapentin binding sites in rodent brain

73P **Löschmann P-A, Kunow M, Lang KW, Turski L, Wachtel H, Jenner P & Marsden CD** Synergism of the AMPA-antagonist NBQX with L-DOPA in the MPTP-treated common marmoset and the unilaterally substantia nigra lesioned rat

74P **Grimwood S, Moseley AM, Carling RW, Leeson PD & Foster AC** Characterisation of the binding of [H]L-689,560, an antagonist for the glycine site on the NMDA receptor, to rat brain membranes

75P **Harvey J, Frenguelli BG, Sunter DC, Watkins JC & Collingridge GL** The actions of 1S,3R-ACPD, a glutamate metabotropic receptor agonist, in area CA1 of rat hippocampus

76P **Chen Y & Stone TW** Release of endogenous adenosine and related purines from the rat hippocampus *in vivo*

77P **McGlynn H, Healy N & Ryan MP** Effects of gentamicin on glucose transport, transepithelial resistances and cell viability in established renal cell lines

78P **Clark KL, Robertson MJ & Drew GM** Effects of the non-peptide angiotensin receptor antagonist, DuP 753, on basal renal function and on the renal effects of angiotensin II in the anaesthetised dog

79P **Hussain JF, Brown CM, Kendall DA, MacKinnon A & Wilson VG** The activity of a crude extract of bovine adrenal medulla at α-adrenoceptors and a non-adrenoceptor, imidazoline, binding site can be accounted for by adrenaline

80P **Hussain JF, Brown CM, Kendall DA, MacKinnon A & Wilson VG** A crude extract of bovine lung displaces ligands from α_2-adrenoceptors and a non-adrenoceptor, imidazoline binding site

81P **McQueen DS** Effects of adenosine on rat carotid body chemoreceptors

82P **Milligan G, Mullaney I, Wakelam MJO & Mitchell F** Production and characterisation of antisera directed against the $G_{11\alpha}$ gene product

83P **Hu DE & Fan T-PD** Synergistic interaction between bradykinin and interleukin-1 in angiogenesis

84P **Buchan KW & Martin W** Inhibition of thrombin-induced increases in endothelial permeability by forskolin and atriopeptin II

85P **Ring RC, Seldon PM, Perkins RS, Dent G, Barnes PJ & Giembycz MA** Identification and partial characterisation of a receptor for platelet activating factor on guinea-pig peritoneal macrophages

86P **Dent G & Barnes PJ** Platelet activating factor stimulates a pertussis toxin-sensitive GTPase activity in guinea-pig eosinophil membranes

87P **Zembowicz A, Salvemini D, Gryglewski RJ & Vane JR** Pyocyanin or methylene blue potentiates the antiaggregatory action of sodium nitroprusside by a mechanism involving the release of nitric oxide

88P **Walker TR & Watson SP** Effect of Ro 31-8220, a novel PKC inhibitor, on human platelet phosphorylation and secretion

89P **Shaw N, Foulkes R, Morgan A, Stephens S, Higgs G & Bodmer M** Investigation of the efficacy of a panel of anti-human TNF antibodies in the conscious rabbit model

90P **Armstrong RA, Marr C & Merle P** PGE_2 potentiates plasma exudation in rabbit skin by two different mechanisms

91P **Berry CN & M'barga-Owono D** Effects of the new antihistamine SL 85.0324 on histamine-induced eicosanoid release from the isolated perfused guinea-pig lung

92P **Peers SH, Duncan GS & Flower RJ** Strain differences in the antibody response: importance of corticosteroids

93P **Greenstein BD, Mander J & Bridges E de F** Oestrogen receptors and role of aromatisation in testosterone-induced inhibition of thymus development in female rats

94P **Watson CD, Fone KCF, Bennett GW & Dickinson SL** Measurements of thyrotrophin releasing hormone and the thyroid axis in rats during learning on a water maze: effects of scopolamine

95P **Hadley AJ, Flack JD & Buckingham JC** Selective phosphodiesterase inhibitors and the release *in vitro* of corticotrophin (ACTH) and luteinising hormone

96P **Guild SB** The effect of adenosine 3',5'-cyclic monophosphate and guanine nucleotides upon calcium-evoked ACTH secretion from electrically permeabilised AtT-20 cells

POSTER COMMUNICATIONS

97P **Barfaraz AR & Pollock D** The effects of drugs and de-medullation on pressor responses to preganglionic nerve stimulation in the pithed rat

98P **Daly CJ & Wilson VG** Sympathetic neuroeffector transmission in the rabbit isolated saphenous vein

99P **Liu SF, Crawley DE, Evans TW & Barnes PJ** Endogenous nitric oxide inhibits adrenergic neurogenic vasoconstriction in guinea-pig pulmonary artery

100P **Darroch SA, Choo LK & Mitchelson F** Difference in the response of two agonists at inhibitory prejunctional muscarinic receptors in the rabbit ear artery

101P **Bulloch JM, Montgomery I & McGrath JC** Pharmacological and electron microscopical examination of a blood vessel exhibiting co-transmission

102P **MacLean MR, Templeton AGB & McGrath JC** Effect of endothelin-1 on human umbilical and placental blood vessels: a comparison with 5-HT

103P **Pérez-Vizcaino, Duarte J, Delpón E & Tamargo J** Effects of flecainide in vascular smooth muscle

104P **Hamilton JT & Lyles GA** Interactions of phenelzine with vasoconstrictor and vasodilator mechanisms in rat aorta

105P **Templeton AGB, McGrath JC & Whittle MJ** Interaction between extracellular calcium, 5-HT, thromboxane A_2 and oxygen in the human umbilical artery

106P **Docherty JR & Borton M** Further investigations of contractile responses to 5-HT in the human saphenous vein

107P **Mylecharane EJ & Martin GR** The apparent heterogeneity in functional 5-HT_2 receptors revealed by antagonists may be species-related

108P **Christie MI & Smith GW** Fenoldopam (SKF 82526) is a low efficacy agonist at the 5-HT_2 receptor

109P **McGrath JC, Templeton AGB, Macmillan JM & Alves C** Demonstration of post-junctional α_2-adrenoceptors in perfused rabbit ear artery

110P **Nally JE & JC McGrath** Modulation of post-junctional α_2-adrenoceptors in the rabbit distal saphenous artery

111P **Osman IA & Naylor IL** Perfused ovine renal glomerular vasculature: a model to study the nature of structural, myofibroblast contractility

112P **Thompson JS, Wanstall JC & Morice AH** The relaxant effects of atrial natriuretic peptide and nitroprusside on pulmonary arteries from rats with monocrotaline-induced pulmonary hypertension

113P **Furman BL & Sneddon P** Lack of effect of long-term streptozotocin diabetes on vascular responsiveness of the rat mesenteric bed *ex vivo*

114P **Demiryürek AT, Wadsworth RM, Kane KA & Peacock A** Comparison of the effects of hypoxia and contractile agonists on human isolated pulmonary artery

115P **Lyles GA & Chalmers J** Metabolism of aminoacetone by semicarbazide-sensitive amine oxidase activity in human umbilical artery

116P **Ramcharan M, Redfern WS & Spedding M** Reistance of cholinergic vasodilatation to ischaemia of the rat mesenteric vascular bed *in vivo* and *in vitro*

117P **Mitchell JA, Sheng H, Warner TD, Pollock JS, Förstermann U & Murad F** Relationship between endothelial cell activation and subsequent NO synthase activity: inhibition by oxygen derived free radicals

118P **Alexander B, Mathie RT, Ralevic V & Burnstock G** Selective purinergic regulation of nitric oxide release

119P **MacLean MR & McGrath JC** The effect of extracellular [Ca^{2+}] reduction, nifedipine, ryanodine and L-NAME on *in vitro* hypoxic pulmonary vasoconstriction in the rabbit

120P **Sunman W, Hughes AD & Sever PS** Does captopril vasodilate by mechanisms other than by ACE inhibition?

121P **Clapham JC, Beerahee A, Longman SD, Mead EA, Stubbs D, Trail BK, Whitehouse SK & Hamilton TC** Effects of repeat oral administration of the potassium channel activator, BRL 38227, in spontaneously hypertensive rats

122P **Widdop RE, Gardiner SM, Kemp PA & Bennett T** Cardiac haemodynamic effects of L-arginyl-L-phenylalanine in conscious rats

123P **Gardiner SM, Kemp PA, Bennett T, Bose C, Foulkes R & Hughes B** Involvement of β_2-adrenoceptors in the regional haemodynamic responses to bradykinin in conscious rats

124P **Einstein R, Abdul-Hassain N, Wong TW, Richardson DP & Matthews R** Cardiovascular responses to dopexamine in anaesthetised and conscious dogs

125P **Gardiner SM, Kemp PA & Bennett T** Effects of captopril on regional haemodynamic responses to angiotensin I in conscious rats

126P **Akers JS, Hilditch A, Robertson MJ & Drew GM** Does a relationship exist between the antihypertensive action and the angiotensin receptor antagonist activity of DuP 753 in conscious rats?

127P **Crabbe DST, Redfern PH, Woodward B & Williams KI** Captopril enhances glucose tolerance in spontaneously hypertensive rats

128P **DiFrancesco GF & Tarnus C** The hypotensive effects of a new renin inhibitor in the dog

129P **Grant MH & Rodger IW** Glutathione status in sensitised guinea-pig lungs

130P **Aitken S & McCaig DJ** Effect of cromakalim on vagally-mediated airway constriction in the guinea-pig *in vivo*

131P **Miura M, Belvisi MG, Stretton CD & Barnes PJ** Role of K^+ channels in the modulation of cholinergic neural responses in airway smooth muscle

132P **Miura M, Belvisi MG & Barnes PJ** Modulation of excitatory non-adrenergic, non-cholinergic constrictor responses in guinea-pig bronchi by bradykinin

133P **Meini S, Evangelista S, Geppetti P & Manzini S** Lipoxin A_4 evokes airway motor responses by activating sensory fibres: functional and neurochemical evidence

134P **Vila E & Tabernero A** Different effects of neuropeptide Y on noradrenaline and nerve stimulation responses in the rat anococcygeus muscle

135P **Anderson K & Pollock D** The effects of ethanol on responses of the rat isolated vas deferens to field stimulation

136P **Santicioli P, Maggi CA, Theodorsson E. Giuliani S & Giachetti A** Functional innervation of the guinea-pig renal pelvis: role of sensory neuropeptides

137P **Sheng H** Effect of LY 83583 on the reponse to NANC nerve stimulation in rat anococcygeus and bovine retractor penis muscles

138P **Lawrence RA & Jones RL** Incomplete inhibition by atropine of the neuronally-mediated contractile action of cicaprost on the guinea-pig ileum

139P **Alyami AM, Patel IA & Wood D** Inhibition of intestinal GABA responses by electrical field stimulation

140P **Pertwee RG, Stevenson LA, Elrick DB. Mechoulam R & Corbett AD** Inhibitory effects of enantiomeric cannabinoids in preparations of mouse vas deferens and of myenteric plexus-longitudinal muscle of guinea-pig small intestine

141P **Woods M & Baird AW** Increased responsiveness of guinea-pig gallbladder to kinins

142P **Maubach K, Patel M & Spraggs CF** Interaction of gastrin/cholecystokinin agonists and antagonists on guinea-pig gall-bladder

143P **Gaynor SC, Wardle KA, King BF & Sanger GJ** Location of putative 5-HT_4 receptors in rat oesophagus

144P **Martin MI, Alfaro MJ, Goicoeachea C & Colado MI** *In vitro* study of the effect of salmon calcitonin upon opioid receptors

145P **Patacchini R, Maggi CA & Giachetti A** Effect of newly developed tachykinin agonist and antagonists on the guinea-pig isolated gallbladder

146P **Boyle SJ, Howson W & McKnight AT** An examination of the selectivity of a new non-peptide tachykinin antagonist

147P **Barr AJ, Nimmo A & Watson SP** The presence of NK_3 tachykinin receptors on rat uterus

148P **Benito-Orfila MA, Nandha KA & Bloom SR** Actions of various neuromedin U fragments on the smooth muscle of the isolated rat uterus

149P **Flowers JM, Hall JM & Morton IKM** NK_1 and NK_2 neurokinin receptors characterized in the rat isolated urinary bladder preparation

150P **Whiting RL, Perkins LA, Lee H-J & Eglen RM** Comparison of 5-HT receptors in oesophageal muscularis mucosae in various species, *in vitro*

151P **Tuladhar BR, Costall B & Naylor RJ** Putative 5-HT_4 receptor involvement in the relaxations induced by 5-HT in the rat ileum

152P **McLaughlin DP, Fulton JD, MacDonald E, Scott PJW & MacDonald A** β-adrenoceptors mediating relaxation in human colon *in vitro*

153P **Quinn P, Miner WD & Wallis RM** Cholecystokinin-induced intestinal motility in the anaesthetised dog: a novel model for evaluating inhibitors of intestinal motility

154P **McRitchie B & Merner P** The actions of some muscarinic antagonists on digestive jejunal motility in the conscious dog

155P **Growcott JW, Wilson C, Holloway B & Mainwaring S** Evaluation of ICI D7114, a putative stimulant of brown adipocytes, on histamine pre-contracted guinea-pig ileum

156P **Fraser S & Spedding M** RS-87476, a novel neuroprotective agent, antagonises the effects of calcium channel activators in guinea-pig taenia

157P **O'Malley KE, O'Donnell E & Baird AW** Neuronal involvement in cholinergic regulation of ion transport in rat colon

158P **Yurdakos E & Webber SE** Endothelin-1-induced inhibition of submucosal gland secretion and epithelial albumin transport is mediated partly by activation of protein kinase C

159P **Arkle S, Goodman PA & Hill MP** Characterisation of purinoceptors in rat parotid salivary glands

160P **Everett CM. Hall JM, Mitchell D & Morton IKM** Contrasting properties of bradykinin receptor subtypes mediating contractions of the rabbit and pig isolated iris sphincter pupillae preparations

161P **Wickenden AD, Pomfret JE, Ellis K, Todd MH, Zuzack J & Grant TL** Comparison of the effects of K^+ channel blockers and cromakalim in guinea pig detrusor and rat mesenteric artery

162P **Wickenden AD & Ellis K** The role of K^+-channels in the mechanism of action of isoprenaline: comparison betweeen guinea-pig trachea and rat mesenteric artery

163P **Davies MP, McCurrie JR & Wood D** Comparative effects of four potassium channel openers on the rat ileum

164P **Downing SJ & Hollingsworth M** Cross-tolerance between salbutamol and cromakalim in the rat uterus *in vivo*

165P **Dunn PM, Davis CM, Ganellin CR, Haylett DG, Morgan JM & Jenkinson DH** Potassium channel blocking activity of dequalinium analogues in guinea-pig hepatocytes and rat sympathetic neurones

166P **Sullivan JP, Ko C, Shearer BG & Burch RM** NPC 15437, a selective inhibitor of protein kinase C, inhibits T cell activation *in vitro* and *in vivo*.

167P **Wise H, Bridge DA & Hallam TJ** The activity of octimibate at prostanoid receptors on human monocytes

168P **Duwiejua M, Zeitlin IJ, Chapman J, Waterman PG & Provan GJ** The anti-inflammatory actions of mansumbinoic acid and mansumbinone, two novel triterpenes from *Commiphora incisa*

169P **Rotondo D, Rae MG, Milton AS & Dutta-Roy AK** Interleukin-1α inhibits the binding of prostaglandin E_2 to macrophages/monocytes

170P **Herd CM, Shoupe TS & Page CP** Effect of PF 5901 on PAF-induced airway responses in neonatally immunised rabbits

171P **Minshall E & Page C** Neonatal immunization of rabbits using house-dust mite allergen (Der p I) induces airway hyperresponsiveness

172P **Frodsham G, Dickinson K, Hayes SL, Jones RB & Yates DB** Effect of flosequinan and BTS 53 554 upon isoenzymes of phosphodiesterase (PDE) isolated from guinea-pig ventricle using Mono Q FPLC

173P **Jones RB, Frodsham G, Dickinson K & Foster GA** Effect of flosequinan on cGMP and cAMP levels in ischaemic and non-ischaemic rat ventricle

174P **Dickinson K, Frodsham G & Jones RB** Flosequinan and its major metabolite, BTS 53 554, do not interact with the L-type calcium channel from guinea-pig ventricle

175P **Patmore L, Duncan GP, Kenny BA & Spedding M** RS-87476, a novel neuroprotective agent, inhibits veratrine-induced calcium overload in embryonic chick cardiac myocytes

176P **Petty MA & Dow J** Subcutaneously administered MDL 73404: its distribution and effects on infarct size

177P **Barr SM, Pye M, Cobbe SM** A rabbit model of heart failure induced by chronic rapid ventricular pacing, and the associated electrophysiological effects

178P **Valenzuela C, Delpón E, Tamargo J & Bennett PB** Block of single cardiac sodium channels by quinidine

179P **Delpón E, Valenzuela C & Tamargo J** Electrophysiological effects of CRE-1087, a new antiarrhythmic drug, in guinea-pig papillary muscles

180P **O'Brien HL & Chess-Williams R** Ischaemia- and reperfusion-induced arrhythmias in isolated hearts with enhanced α-adrenoceptor responsiveness

181P **Adams D, Cooper S, Lad N & MacKenzie I** The use of *in vitro* and *in vivo* rabbit hearts for studies of the role of ATP-dependent potassium channels during ischaemia

182P **Clanachan AS, Gerencer RZ & Finegan BA** Cardiovascular effects of A_1 and A_2-selective agonists *in vivo*

183P **Foulkes R, Shaw S & Bodmer M** Cardiodepressant effect of rec huTNF in isolated rat atria

184P **Cottney JR, Bruin JC, Shahid M, Spiers I, Walker GB, Winslow E & Nicholson CD** Org 20494, a cardiotonic agent with a novel mechanism of action

185P **Steele DS, Smith GL & Miller DJ** Effects of Org 30029 on the development of rigor tension in triton-treated ventricular trabeculae of rat

186P **McIntosh MA, Kane KA & Parratt JR** The effects of opioid peptides on cardiovascular function and sympathetic neurotransmission in rats

187P **Hill RJ, Grant AM, Dessingue OC, Harris AL & Ezrin AM** Ipazilide, a new antiarrhythmic drug: inhibition of [^{3}H]-batrachotoxin binding to cardiac sodium channels and effects on papillary muscle contractility

188P **Vatanpour H, Rowan EG, Harvey AL & Gopalakrishnakone P** The effects of the scorpion venom (*Buthus tamulus*) on neuromuscular transmission

189P **Braga MFM, Rowan EG & Harvey AL** Reversal by cysteine of the cadmium-induced block of neuromuscular transmission

190P **Marshall DL, Harvey AL & Hollecker M** Structure/activity relationships in the facilitatory actions of the dendrotoxins

191P **Pekiner C & McLean WG** The action of ponalrestat on neurofilament phosphorylation

192P **Pemberton K, Prior C & Marshall IG** Exposure to potassium chloride produces a vesamicol-sensitive increase in quantal size at the rat neuromuscular junction

193P **Wilson A, O'Keefe P, O'Mahony D & Barry M** Zidovudine does not impair neuromuscular transmission in the rat phrenic nerve diaphragm preparation

194P **Odber J, Gillespie JS, Moore MR & Goldberg A** The effects of haem synthesis blocking agents on the responses of some rat isolated nerve/muscle preparations

195P **Odber J, Gillespie JS, Moore MR & Goldberg A** Differential effects of haem synthesis blocking agents on erythrocytic, hepatic and neural haemoprotein content

196P **Cowan A, Daniel JL & Ryan RR** Effects of a bombesin analogue on neuromedin B- and bombesin-induced calcium flux in the small cell lung carcinoma line NCI-H345

197P **Sugrue MF, Mallorga P, Schwam H, Baldwin JJ & Ponticello GS** On the preclinical pharmacology of L-685,393 and MK-507, two topically effective ocular hypotensive carbonic anhydrase inhibitors

198P **Lamikanra A, Ramasamy A & Keysell GR** The effect of time and diffusion cell receptor fluid on esterase activity in hairless mouse skin *in vitro*

199P **Campbell D, Nairn A, Willett B, Vass M, Scott A, MacDonald C & Grant MH** Expression of differentiated hepatic function in SV40-immortalised rat liver cells

200P **Duthie SJ, Melvin WT & Burke MD** The toxicity of cyclophosphamide in HepG2 hepatoma cells and cultured rat hepatocytes

201P **Smith AD & Muir TC** The involvement of nitric oxide in neuronally mediated relaxation in the rabbit distal colon

202P **Nixon GF, Hamilton CA, Wadsworth RM, Pasanisi F, Pyne NJ & Reid JL** Noradrenaline and endothelin coupling to IP formation in cultured aortic cells from spontaneously hypertensive rats

203P **Sharifi AM & Pollock D** A comparison of the effects of isoprenaline, acetylcholine and isobutylmethylxanthine on noradrenaline-induced tone and PI hydrolysis in rat aorta *in vitro*

204P **Kelly M & Keenan AK** Investigation of the effect of protein kinase C activation on ANF-stimulated cGMP production in $LLCPK_1$ cells

205P **Bushfield M, MacDonald FM, Morris NJ & Houslay MD** Effects of amylin and CGRP on the adenylate cyclase/cyclic AMP system in rat hepatocytes

206P **Berry JL, Elliott KRF, Foster RW & Small RC** Cyclic nucleotide phosphodiesterase isoenzymes (PDE) in guinea-pig tracheal smooth muscle

207P **Patel S & Freedman SB** Stimulation of *in vivo* accumulation of inositol monophosphates in mouse CNS by lithium, pilocarpine and L-658,903

208P **Jenkinson S, Patel N, Challis RAJ & Nahorski SR** Inhibition of muscarinic receptor-stimulated inositol polyphosphate accumulation by lithium in rat cerebral cortex and striatum

209P **Alexander SPH, Hill SJ & Kendall DA** Muscarinic receptor stimulation of particulate phosphoinositidase C activity from cerebral cortex

210P **White AM, Watson SP & Varney MA** Comparison of solubilised inositol 1,4,5-trisphosphate (IP3) receptors in bovine adrenal cortex and rat cerebellum

211P **Czyrak A, Marsden CA & Kendall DA** Augmentation of adenosine receptor-stimulated cyclic AMP accumulation by 5-HT in guinea-pig brain slices

212P **Morgan AJ, Murray KJ & Challis RAJ** Anomalous effects of isobutylmethylxanthine on cyclic AMP accumulation in SH-SY5Y neuroblastoma cells

213P **Mackinnon MWB, Morrison P & Stone TW** Changes in hippocampal adenosine and baclofen sensitivity in streptozotocin diabetic rats

214P **Goudie AJ & Leathley MJ** Operant "behavioural dependence" studies with chlordiazepoxide in rats: lack of effect of ondansetron

215P **Hasan MY, Sewell RDE & Nicholls PJ** Differential acute action of sodium valproate, vigabatrin, beclamide and aminobeclamide on 5-HTP-induced head twitch

216P **Luscombe GP, Mazurkiewicz SE & Buckett WR** Flumazenil antagonises the anxiolytic effect of chlordiazepoxide, buspirone and dothiepin in the elevated plus-maze

217P **Guild SB & Mackay KB** The effect of guanosine 3,5,-cyclic monophosphate upon secretagogue and calcium-evoked ACTH secretion from AtT-20 pituitary tumour cells

218P **Smith T, Smith SF, Flower RJ & Buckingham JC** Endotoxin and the expression of lipocortins in central and peripheral tissues of the rat

219P **Bratt AM, Costall B, Domeney AM & Kelly ME** The effect of ageing on Stone maze performance and brain levels of choline acetyltransferase

220P **Shek F, Hill SJ & Fone KCF** Distribution of thyrotrophin-releasing hormone-like immunoreactivity and choline acetyltransferase activity in the guinea-pig brain

221P **Kulkarni RS, Creed CL, Fone KCF, Marsden CA & Bennett GW** Distribution and release of acetylcholine in rat brain measured by HPLC with electrochemical detection

222P **Sharkey J & Kelly PAT** Pharmacological characterisation of muscarinic receptors and choline reuptake sites in intracerebral implants of foetal rat basal forebrain

223P **Connick JH, Fox PK & Nicholson CD** Interaction of certain calcium antagonists with sigma binding sites

224P **Wong JCL, Dambisya YM & Chan K** Potentiation of opioid antinociceptive activity by ephedrine and phenylpropanolamine in mice

225P **Sagan S, Charpentier S, Corbett AD, Amiche M, Delfour A, Nicolas P & Kosterlitz HW** Opioid activity of some analogues of dermenkephalin and deltorphin I in binding assays and bioassays

226P **Hawcock AB, Barnes JC & Michel AD** Pharmacological characterisation of angiotensin II-induced depolarisation of the rat isolated superior cervical ganglion

227P **Duggan AW, Hope PJ, Lang CW & Schaible H-G** Antibody microprobes detect immunoreactive substance P over an increased area of the dorsal horn following endopeptidase inhibition

228P **Elliott PJ, Turpin MP & Mason GS** Characterization of neurokinin receptors in the rat nucleus accumbens

229P **Guard S, Zarnegar R, Watling KJ, Quirion R, Takeda Y & Krause JE** ^{125}I-neuropeptide-g and ^{125}I-iodohistidyl-neurokinin A label multiple tachykinin receptors in rat brain

230P **Beresford IJM, Hagan RM & Ireland SJ** The non-peptide tachykinin NK-1 receptor antagonist, CP-96,345, may discriminate species variants in NK-1 receptors

231P **Andersson M, Poat JA, Campbell B, Dockray G & Hughes J** Distribution of CCK receptor subtypes in rat and chick brain

232P **Ebenezer IS & Baldwin BA** Different effects of cholecystokinin (CCK) on the electroencephalogram (EEG) of unanaesthetised rats and sheep

233P **Gupta J & Ceasar PM** Effect of L-365,260 on the release of cholecystokinin-like immunoreactivity from rat nucleus accumbens *in vitro*

234P **Frenguelli BG & Collingridge GL** A grease-gap study of the effects of hypoxia on electrical activity of hippocampal CA1 neurones

235P **Bartrup JT, Addae JI & Stone TW** Purine inhibition is reduced during NMDA stimulation of single pyramidal cells in the rat hippocampal slice

236P **Xiang J, Brammer MJ & Campbell IC** Presynaptic increases in $[Ca^{2+}]_i$ induced by quisqualate and carbachol are temporally different and are mediated by distinct mechanisms

237P **Bashir ZI & Collingridge GL** Reversible homo- and heterosynaptic depression of synaptic transmission induced by the activation of NMDA receptors in the CA1 region of the rat hippocampus

238P **Bagetta G, Corasaniti MT, Nistico G & Bowery NG** Evidence that seizures and neuronal cell death produced by intrahippocampal tetanus toxin in rats can be reduced by CGP 37849 and carbamazepine

239P **Davidson A, Olverman HJ & Butcher SP** [^{3}H]dizocilpine and [^{3}H]CPP binding in the developing rat brain

240P **Martin D, Thompson MA & Nadler JV** The neuroprotective agent riluzole inhibits release of L-glutamate and L-aspartate from slices of hippocampal area CA1

241P **Grimwood S, Wilde GJC & Foster AC** Differential modulation of [^{3}H]glycine and [^{3}H]L-689,560 binding to the glycine site on the NMDA receptor by glutamate site antagonists

242P **Steele JE, Cross AJ & Bowen DM** Ligand binding to the N-methyl-D-aspartate receptor in brains from controls and Alzheimer's disease patients

243P **Gulati J & Foster AC** The binding of [^{3}H]L-689,560, an antagonist of the glycine site on the NMDA receptor, to membranes from post-mortem human cerebral cortex

244P **Scott G, Luscombe GP & Mason R** The effect of BTS 54 505 on NMDA-evoked responses in the rat dorsolateral geniculate nucleus and hippocampus

245P **Rabbani M & Little HJ** A competitive antagonist at NMDA receptors, at low doses, protects against the barbital withdrawal syndrome, but cross-tolerance does not occur

246P **Wright J, Rabbani M & Little HJ** Chronic barbital treatment increases MK801 binding

247P **Larkin JG, Scobie G, Thompson GG, Drennan J & Brodie MJ** Central and peripheral (brain and platelet) benzodiazepine receptors in the rat - effect of diazepam and clobazam

248P **Jagger SM, Barnes NM, Costall B, Domeney AM, Naylor RJ, Smith AG & Smith JA** Lack of effect of intra-accumbens dopamine infusion on 5-HT_3 recognition sites in limbic and cortical areas of rat brain

249P **Coleman J, Lattimer N, Rhodes KF & Saville VL** 5-HT_3- and 5-HT_4-like actions of the enantiomers of zacopride in some *in vitro* functional assays

250P **Bill DJ, Fletcher A, Hallett I & Middlefell VC** The enantiomers of zacopride: correlation of anxiolytic activity with *in vivo* functional 5-HT_3 antagonism in the mouse

251P **Gao B & Cutler MG** Effects of sub-chronic treatment with chlordiazepoxide, buspirone and the 5-HT_3 antagonist, BRL 46470, on the behaviour of mice

252P **Mitchell EA, Pratt JA & Kilpatrick GJ** Effects of the 5-HT_3 receptor agonist mCPBG in the social interaction test of anxiety

253P **Mundey MK, Marsden CA & Fletcher A** Effect of isolation rearing on 5-HT_{1A} autoreceptor function in the dorsal raphé nucleus

254P **Cutler MG & Piper DC** Effects of diazepam and the 5-HT_3 antagonists, granisetron and BRL 46470, on the behaviour of intruder mice

255P **Forster EA & Fletcher A** Evidence supporting an involvement of 5-HT_{1C} receptors in some behavioural effects of DOI, and its apparent facilitation of 5-HT_{1A} receptor activation *in vivo*

256P **Cheetham SC. Frankland ATJ, Prow MR, Butler SA & Heal DJ** RU 24969: characterisation of the locomotor response in mice with isomers of pindolol and propranolol and effects on brain catecholamines and metabolites

257P **Baldwin HA, Blagden S, Snares M, De Souza RJ, Cross AJ & Green AR** Consequences of photochemically-induced ischaemia on 5-HT biochemistry in the rat brain

258P **Lawrence JA, Shirakawa K, Overman HJ, Kelly JS & Butcher SP** Comparison of AMPA and ibotenic acid lesions in rat cortex

259P **Kilpatrick GJ, Hagan RM, Butler A, Burridge J, North PC, Oxford AW & Tyers MB** GR68755, a potent and selective antagonist of 5-HT_3 receptors

260P **Trout SJ & Kruk ZL** Frequency dependent dopamine efflux from the nucleus accumbens in rat brain slices: the influence of autoreceptors

261P **Thomas K, Jenner P & Marsden CD** The relationship between the extent of nigral lesion induced by quinolinic acid and rotational behaviour in rats

262P **Dawson DA, Robinson MJ, Macrae IM, Reid JL & McCulloch H** Reductions in forskolin and D_1-dopamine receptor binding following focal cerebral ischaemia in the rat

263P **Earl CD & Kruk ZL** 6-hydroxydopamine injected into the lateral hypothalamus causes a functional depletion of dopamine in the rat caudate putamen and nucleus accumbens

264P **Armstrong RA, Spencer PSJ & Sewell RDE** Contrasting effects of dopamine D_1 and D_2 receptor agonists on opioid precipitated withdrawal

265P **Balfour DJK & Gerrard L** Attenuation of locomotor stimulant responses to nicotine and amphetamine by selective dopamine receptor antagonists

266P **Heal DJ, Prow MR, Butler SA & Buckett WR** The influence of adrenalectomy on the desensitisation of α_2- and β-adrenoceptors induced by repeated electroconvulsive shock

267P **Nevinson MJ, Gray AM & Sewell RDE** Antagonism of nefopam antinociception by naloxone and RX821002

268P **Jackson HC, Ripley TL & Nutt DJ** Behavioural profile of the non-adrenoceptor idazoxan binding site ligand RX801023 in mice

269P **Hussain JF, Wilson VG & Kendall DA** The effect of guanine nucleotides on [^{3}H]-idazoxan binding sites in bovine cerebral cortex

270P **Sturman G, Freeman F, Jakobson ME, John TH & Nicol S** Effects of histaminergic modulation on body temperature in mice

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271P **Higgins MJ & Asbury AJ** A physiological model of fentanyl pharmacokinetics for various routes of administration

272P **Smith GL, Crichton CA & Steele DS** A novel method of measuring the [Ca^{2+}] within permeabilized smooth and cardiac muscle preparations

ORAL COMMUNICATIONS

In oral communications with more than one author, the first author is the one who intended to present the work

273P **Jones CA & Silvers KM** Methohexitone inhibition of K^+-stimulated release of [^{3}H]noradrenaline from mini-slices of rat cerebral cortex is dependent on K^+ concentration

274P **Siarey RJ, Long SK & Evans RH** Effect of tizanidine on synaptic activity of rat *in vitro* spinal cord preparations

275P **De Paermentier F, Crompton MR, Katona CLE & Horton RW** Brain α_2-adrenoceptor binding sites in antidepressant-treated suicides and controls

276P **Payvandi N, Elliott JM & Heal DJ** Phosphatidylinositol potentiates the effect of amitriptyline on β_1-adrenoceptor number and on noradrenaline-stimulated adenylyl cyclase activity in rat frontal cortex

277P **Taylor SJ, Michel AD & Kilpatrick GJ** The effect of thioperamide on *ex vivo* [^{3}H](R)-α-methylhistamine binding to rat brain histamine H_3 receptors

278P **Taylor SJ & Kilpatrick GJ** Irreversible blockade of peripheral and central histamine H_3 receptors by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline

279P **Baldwin HA, Jones JA, Snares M, De Souza R, Cross AJ, Green AR & Jostell KG** Chlormethiazole is neuroprotective following transient forebrain ischaemia when given as an intravenous infusion to the gerbil

280P **Green AR, De Souza RJ, Williams JL, Murray TK, Cross AJ & Jonsson G** Chlormethiazole prevents methamphetamine-induced neurotoxicity: studies on rat brain 5-HT and dopamine concentrations

281P **Sian J, Dexter DT, Jenner P & Marsden CD** Decrease in nigral reduced glutathione levels in Parkinson's disease

282P **Blunt SB, Jenner P & Marsden CD** Autoradiographic study of striatal D_1 and D_2 dopamine receptors in 6-OHDA-lesioned rats receiving foetal nigral grafts and L-DOPA treatment

283P **Gnanalingham KK, Hunter AJ, Jenner P & Marsden CD** Autoradiographic study of D_1 and D_2 dopamine receptor densities in the basal ganglia of the MPTP-treated common marmoset

284P **Smith LA, Jenner P & Marsden CD** Effect of α-methyl-p-tyrosine (AMPT) on locomotor activity produced by dopamine agonists in MPTP-treated common marmosets

285P **Keegan KD, Pinnock RD, Woodruff GN & Richardson R** The pharmacology of novel neurotensin analogues on neurones in the rat substantia nigra pars compacta, *in vitro*

286P **Harley EA, Woodruff GN, Middlemiss DN & Ragan CI** The pharmacology of dopamine D_2 receptors in rat striatum and the transfected $D_{2\,(444)}$ in CHO cells

287P **Ahlenius S** Stimulation of locomotor activity by blockade of somato-dendritic dopamine D_2 autoreceptors in the rat

288P **Murphy TV & Majewski H** Prejunctional α_1-adrenoceptors in the rat kidney

289P **Abrahamsen J & Nedergaard OA** Role of cyclic AMP in the stimulation-evoked release of noradrenaline from sympathetic neurones in rabbit isolated ear artery

290P **Carnell AJ, Waldron G & Williams RG** Evidence supporting a role for nitric oxide in the vagally evoked inhibition of intragastric pressure

291P **Schultheis AH, Bassett DJP & Fryer AD** Changes in neuronal control of airway smooth muscle after exposure to ozone

292P **Fryer AD & Jacoby DB** Antigen-induced pulmonary M_2 muscarinic receptor dysfunction in guinea-pigs is reversed by heparin and poly-l-glutamate

293P **O'Donnell SR & Anderson GP** Attenuation by formoterol of tracheal plasma exudation by topical bradykinin in rat trachea *in vivo*

294P **Nials AT, Whelan CJ & Vardey CJ** Salmeterol inhibits neutrophil accumulation in rat lung

295P **Morley J, Chapman ID, Foster A, Hoshiko K & Mazzoni L** Effects of (+) and racemic salbutamol on airway responses in the guinea-pig

296P **Garland CJ & McPherson GA** Nitric oxide and acetylcholine hyperpolarize smooth muscle cells in the rat small mesenteric artery by different mechanisms

297P **Duty S & Weston AH** The effect of BRL 38227 on tension and intracellular calcium levels in isolated smooth muscle of bovine trachealis

298P **White RP, Ricca GF, El-Bauomy AM & Wood WB** Identification of capric acid as a potent vasodilator of human and canine basilar arteries

299P **Bevilacqua M, Norbiato G, Baldi G, Chebat E, Bertora P & Vago T** Flosequinan relaxes stretch-induced tone and contractions induced by KCl, phorbol dibutyrate and noradrenaline in isolated human artery and vein strips

300P **Robertson MJ, Middlemiss D, Ross BC, Drew GM, Scopes DIC and Dowle MD** GR117289: a novel, potent and specific non-peptide angiotensin receptor antagonist

301P **Plevin R & Wakelam MJO** The role of PLD catalysed PtdCho hydrolysis in sustained diacylglycerol formation in vasopressin-stimulated A10 vascular smooth muscle cells

302P **Gerwins P & Fredholm BB** ATP and its metabolite adenosine act synergistically to mobilize intracellular calcium via the formation of Ins(1,4,5)P_3 in a smooth muscle cell line

303P **Mackay KB, Kusumoto K & McCulloch J** The neuroprotective effect of the kappa agonist CI-977 in a rat model of focal cerebral ischaemia

304P **Relton JK, Carey F & Rothwell NJ** The neuroprotective actions of lipocortin-1 in cerebral ischaemia in the rat

305P **Black MD, Relton JK, Carey F, Crossman AR & Rothwell NJ** Lipocortin-1 inhibits NMDA receptor-induced neuronal damage

306P **Jones RSG** The role of $GABA_B$ receptors in terminating epileptiform activity in the rat entorhinal cortex *in vitro*

307P **Anderson SMP, Cross AJ, De Souza RJ & Green AR** The IMR-32 human neuroblastoma cell possesses a functional $GABA_A$ receptor

308P **Alexander B, Aslam M & Benjamin IS** Brain indole amine metabolism during portal-systemic encephalopathy

309P **Colquhoun L, Holden-Dye L & Walker RJ** Neuronal bungarotoxin is a potent antagonist at the nicotinic receptor on the somatic muscle cells of the parasitic nematode *Ascaris suum*

310P **Cunha RA & Sebastião** Evidence that ecto-5'-nucleotidase activity in rat hippocampal but not in cerebral cortical slices is associated with the cholinergic nerve terminals

311P **Parkinson FE, van der Ploeg I & Fredholm BB** Coupling of adenosine A_1 and A_2 receptors to G-proteins as studies with autoradiography

312P **Murrin RJA & Boarder MR** Neuronal P_2-purinergic (or nucleotide) receptors: action of adenosine 5'-0-(3-thiotriphosphate) on PC12 cells

313P **Hadley AJ, Flack JD & Buckingham JC** Potentiation of cyclic AMP mediated corticotrophin (ACTH) release by denbufylline *in vivo* and *in vitro*

314P **Pinnock RD, Boden PR & Woodruff GN** Selective CCK_A and CCK_B receptor ligands reveal two populations of CCK receptors in the ventromedial hypothalamus of the rat *in vitro*

315P **Bak M, Poulsen LB, Shalmi M, Petersen JS & Christensen S** Effects of enalaprilat on the renal adaptations to constant furosemide infusion in conscious rats

316P **Christensen S, Lunau H, Bak M, Petersen JS & Shalmi M** Effects of constant infusion of diuretics on 24-hour lithium clearance in conscious chronically instrumented rats

317P **Huang C, Davis G & Johns EJ** Effect of recombinant human erythropoietin on the renal vasculature of the rat

318P **Munavvar AS & Johns EJ** Characteristics of α-adrenoceptors in the rat renal vasculature

319P **Burden DT, Hawthorne YM & Waterfall JF** Effects of Ro 31-5460 on renal ischaemia and reperfusion damage in rats

320P **Rees SA & Curtis MJ** Mechanism of antifibrillatory action of UK-66914: studies in rabbit

321P **Greengrass PM, Russell MJ & Wyllie MG** Abanoquil: a novel α_{1A}-adrenoceptor antagonist

322P **Lad N, Burden DT, Budd JM, Crackett PH, Kirtland SJ, Manning AS, Weir A & Waterfall JF** Reduction of reperfusion-induced myocardial tissue necrosis by Ro 31-5460 in anaesthetised rabbits and dogs

323P **McMurdo L, Sessa WC, Thiemermann C & Vane JR** Ischaemia and reperfusion injury potentiates the vasoconstrictor effects of endothelin-1 in the isolated perfused heart of the rat

324P **Christiansen EH, Böttcher M, Wiggers HS, Tian R & Andreasen F** Effect on cardiac contractile pattern of early and late angiotensin converting enzyme inhibition after myocardial infarction in rats

325P **Schulz R, Nava E, Moncada S** Endotoxin and cytokines induce a Ca^{2+}-independent nitric oxide synthase in myocardium and isolated myocytes

326P **Crook TJ, Kitchen I & Hill RG** Differences in sensitivity of antinociceptive effects of selective δ-opioid agonists to antagonism by naltrindole in 25-day-old rats

327P **Andreev N & Dray A** Morphine-induced depression of peripheral nociceptor activity following inflammatory hyperalgesia: studies in an isolated skin/nerve preparation of adult rats

328P **Andreeva LA & Rang HP** Effects of bradykinin and prostaglandins on CGRP release from spinal cord slices

329P **Chan K, Dambisya YM & Wong CL** The effects of sympathomimetic agents on the disposition of codeine and morphine in mice

330P **Ali Z, Barasi S & Wu G** Evidence to suggest that the antinociceptive action of intrathecally applied 5-HT in the rat spinal cord is mediated by non-5-HT_3 receptors

331P **Wallis DI & Wu J** Inhibition by 5-HT of ipsilateral and contralateral fast and slow spinal reflexes in the neonate rat

332P **Hawkins NS, Clark KC & Evans RH** Effect of concanavalin A on excitatory amino acid-induced depolarization of dorsal root C fibres

333P **Pook PC-K, Jane DE, Sunter DC, Udvarhelyi PM & Watkins JC** New willardiine analogues with potent stereoselective actions on mammalian spinal neurones

334P **Blake JF, Jane DE & Watkins JC** Action of willardiine analogues on immature rat dorsal roots

335P **Zeman S & Lodge D** Barbiturates selectively reduce depolarising responses to kainate rather than those to AMPA in neonatal rat spinal cord *in vitro*

336P **Fedele E & Foster AC** Changes in striatal extracellular amino acids measured by intracerebral dialysis following injection of a neurotoxic dose of quinolinate into the rat striatum

337P **Harrison PS & Kilpatrick IC** Some inhibitors of Na^+-dependent quisqualate accumulation by rat hippocampal slices

338P **Porter RHP, Roberts PJ & Briggs RSJ** Modulation of [^{3}H]CPP binding by NMDA receptor complex ligands

339P **Thorn L, Hutson PH, Bristow LJ & Tricklebank MD** The stimulation of dopamine metabolism by dizocilpine (MK-801) is blocked by R(+)-HA-966

340P **Bowie D & Smart TG** Functional diversity of non-NMDA receptors expressed from avian and mammalian brain mRNA-injected *Xenopus laevis* oocytes

341P **Sizer AR & Roberts MHT** Epileptiform discharge mediated exclusively by non-NMDA receptors is depressed by 5-HT in rat entorhinal cortex *in vitro*

342P **Motani AS, Salvemini D, Änggård EE & Ferns GAA** Insulin-like growth factor-1 potentiates platelet aggregation induced by collagen, thrombin or ADP

343P **Sessa WC, Zembowicz A, Macarthur H, Southan GJ, Walsh DT, Hecker M & Vane JR** Cultured endothelial cells convert N^G-hydroxy-L-arginine to nitric oxide

344P **Antunes E, Giraldelo CM, Cirino G & de Nucci G** Effects of EDRF synthesis inhibitors on rat paw oedema

345P **Zembowicz A & Vane JR** Toxic shock syndrome toxin-1 induces NO-synthase in a macrophage-like cell line

346P **Rothwell NJ, Strijbos PHLM, Hardwick AJ, Relton JK & Carey F** Inhibition of cytokine action by lipocortin-1 (annexin-1): implications for mechanisms of action

347P **Al-Qatari M, Leighton IA & Taberner PV** Chronic ethanol stimulates lipogenesis in brown adipose tissue of normal and obese diabetic CBA/Ca mice

348P **Bascal ZA, Moonga BS, Zaidi M & Dacke CG** Contrasting effects of elevated extracellular calcium and a calcium ionophore on isolated neonatal rat and quail medullary bone osteoclasts

349P **Scott CM, Bunce KT & Spraggs CF** Investigation of the 5-HT receptor types controlling the short-circuit current response in guinea-pig ileal mucosa

350P **Quilley J & McGiff JC** Role of cyclooxygenase in tachyphylaxis to the vasoconstrictor effects of arachidonic acid in the perfused kidney of the rat

POSTER COMMUNICATIONS

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ERRATUM

The title of 16P, published in Volume 102 Proceedings Supplement, March 1991, should have been "Agonist and antagonist actions of sumatriptan at histamine H_1 receptors" by SJ MacLennan, MA Turner, DJ Prentice & GR Martin